(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 July 2010 (15.07.2010)

PCT

(10) International Publication Number
WO 2010/080835 A1

(51) International Patent Classification:
C07K 17/00 (2006.01)

(21) International Application Number:
PCT/US2010/020259

(22) International Filing Date:
6 January 2010 (06.01.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/142,718	6 January 2009 (06.01.2009)	US
61/153,279	17 February 2009 (17.02.2009)	US
61/170,856	20 April 2009 (20.04.2009)	US

(71) Applicant *(for all designated States except US)*: **CUREMARK LLC** [US/US]; 411 Theodore Fremd Avenue, Suite 206 South, Rye, New York 10580 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FALLON, Joan M.** [US/US]; 1180 Midland Avenue, Bronxville, New York 10708 (US). **HEIL, Matthew** [US/US]; 47 Timber Lake Road, Sherman, Connecticut 06784 (US). **FALLON, James J.** [US/US]; 11 Wampus Close, Armonk, New York 10504 (US).

(74) Agent: **LAVOIE, Teresa A.**; Fish & Richardson P.C., P.O. Box 1022, Minneapolis, Minnesota 55440-1022 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT OR THE PREVENTION ORAL INFECTIONS BY E. COLI

(57) Abstract: Compositions and methods for treating or preventing *E. coli* infections are provided. The compositions can be formulated as pharmaceutical compositions or as disinfectants, sanitizers, detergents or antiseptics, and can be used to eradicate or reduce *E. coli* populations and thereby treat or prevent infection by *E. coli*. The compositions include one or more digestive enzymes, e.g., one or more protease, lipases, and amylases. Methods of use of the compositions are also provided.

WO 2010/080835 A1

COMPOSITIONS AND METHODS FOR THE TREATMENT OR THE PREVENTION oral INFECTIONS by E. COLI

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119 to U.S. Provisional Applications Ser. Nos. 61/142,718, filed January 6, 2009; 61/153,279, filed February 17, 2009; and 61/170,856, filed April 20, 2009, the contents of all of which are hereby incorporated by reference in their entirety.

TECHNICAL FIELD

This disclosure relates to compositions, including pharmaceutical compositions such as antibiotic compositions, and methods of using the same for treating or preventing *E. coli* infections in humans and other animals. This disclosure also relates to compositions, such as disinfectants, sanitizers, antiseptics, and detergents, and methods of using the same for eradicating or reducing the presence of *E. coli*.on surfaces, including inanimate and biological surfaces (e.g., skin, wounds), and/or for attenuating the infectivity of *E. coli* in order to prevent and/or to reduce the spread of *E. coli* infections.

BACKGROUND

Escherichia coli (*E. coli*) is a type of bacteria that can cause severe human illness. While there are many types of *E. coli* bacteria, only certain types cause food borne illness. Hundreds of harmless strains of *E. coli* can be found widely in nature, including the intestinal tracts of humans and other warm-blooded animals. Disease-causing strains, however, are a frequent cause of both intestinal and urinary-genital tract infections.

In 1982, scientists identified the first harmful food borne strain of *E. coli* in the United States. The disease-causing food borne *E. coli* most commonly found in this country is called O157:H7. Cattle are the main sources of *E. coli* O157:H7, but these bacteria also can be found in other domestic and wild mammals. *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine.

Several different strains of harmful *E. coli* can cause diarrheal disease. Particularly dangerous types of *E. coli*, such as *E. coli* O157:H7, produce one or more kinds of toxins (poisons) called Shiga toxins. Shiga toxins can severely damage the lining of intestines and kidneys. These types of bacteria are called Shiga toxin-producing *E. coli* (STEC). STEC often causes bloody diarrhea and can lead to kidney failure in children or in people with weakened immune systems. STEC is also refered to as verocytotoxic E. coli (VTEC) or enterohemorrhagic E. coli (EHEC); these all refer generally to the same group of bacteria. The most commonly identified STEC in North America is E. coli O157:H7 (often shortened to *E. coli* O157 or even just "O157").

Enterotoxigenic *E. coli* (ETEC), which produce a different toxin, can cause diarrhea. These strains typically cause so-called travelers' diarrhea because they commonly contaminate food and water in developing countries. Enteropathogenic *E. coli* (EPEC) cause persistent diarrhea (lasting 2 weeks or more) and are more common in developing countries where they can be transmitted to humans through contaminated water or contact with infected animals. Other types of *E. coli*, including O26:H11 and O111:H8, also have been found in the United States and can cause disease in people.

E. coli infection is characterized by severe cramping (abdominal pain) and diarrhea that is initially watery but becomes grossly bloody. Occasionally vomiting occurs. Fever is either low-grade or absent. The illness is usually self-limited and lasts for an average of 8 days. Some individuals exhibit watery diarrhea only.

Hemorrhagic colitis is diagnosed by isolation of *E. coli* of serotype O157:H7 or other verotoxin-producing *E. coli* from diarrheal stools. Alternatively, the stools can be tested directly for the presence of verotoxin. Confirmation can be obtained by isolation of *E. coli* of the same serotype from the incriminated food.

In addition to E. coli O157, many other kinds (called serogroups) of STEC cause disease. These other kinds are sometimes called "non-O157 STEC." *E. coli* serogroups O26, O111, and O103 are the non-O157 serogroups that most often cause illness in people in the United States.

People of any age can become infected with STEC. Very young children and the elderly are more likely to develop severe illness and hemolytic uremic syndrome (HUS) than others, but even healthy older children and young adults can become seriously ill. The symptoms of STEC infections vary for each person but often include severe stomach cramps, diarrhea (often bloody), and vomiting.

Around 5 to 10% of those who are diagnosed with STEC infection develop a potentially life-threatening complication known as hemolytic uremic syndrome (HUS). Clues that a person is developing HUS include decreased frequency of urination, feeling very tired, and losing pink color in cheeks and inside the lower eyelids. Persons with HUS should be hospitalized because their kidneys may stop working and they may develop other serious problems. Most persons with HUS recover within a few weeks, but some suffer permanent damage or die.

STEC live in the guts of ruminant animals, including cattle, goats, sheep, deer, and elk. The major source for human illnesses is cattle. STEC that cause human illness generally do not make animals sick. Other kinds of animals, including pigs and birds, sometimes acquire STEC from the environment and may carry or spread it.

SUMMARY

This disclosure relates to the prevention and/or treatment of *E. coli* infections, including antibiotic-resistant and virulent *E. coli* infections, such as STEC, ETEC, or EPEC, with the use of a pharmaceutical composition comprising one or more digestive enzymes, such as pancreatic or other digestive-track enzymes (e.g., porcine pancreatic enzymes) or plant-, fungal-, or microorganism-derived enzymes, that break down components of food. As used herein, a pharmaceutical composition can be used for human or veterinary indications. Accordingly, the pharmaceutical compositions can be useful for prophylactic and/or therapeutic treatment of human or other mammalian populations (e.g., pig, horse, cow, sheep, goat, monkey, rat, mouse, cat, dog) or of bird populations (e.g., duck, goose, chicken, turkey).

The pharmaceutical compositions can be used on their own, and/or in combination with other antibacterial or antibiotic (e.g., *anti-E. coli*) regimens, and/or with other therapeutic or antibiotic agents post-infection to treat *E. coli* infections.

Also provided herein are bacteriocidal and/or bacteriostatic compositions comprising one or more digestive enzymes for use as or in disinfectants, sanitizers, detergents, and antiseptics, e.g., in hospitals, nursing homes, nurseries, daycares, schools, work environments, food service settings, public transportation and restroom facilities, to reduce, attenuate, and/or destroy *E. coli* present in such settings. The surfaces treated with the described compositions can be large (e.g., operating room tables, doors, changing tables, ventilation systems) or small (e.g., medical devices, door handles); inanimate or non-living (tables) or living tissue (hands, e.g., detergents for hand-washing; wounds, e.g., surgical wounds or wounds resulting from accidents/trauma). The compositions can thus be useful to treat surfaces to reduce or eradicate *E. coli* thereon, or to attenuate or reduce the infectivity of *E. coli*, and thereby prevent or reduce the spread of *E. coli*.

Accordingly, it is an object of the present disclosure to provide a method for the treatment or prevention of *E. coli* infection in a bird or a mammal, comprising administering to the bird or mammal a therapeutically effective amount of a pharmaceutical composition comprising one or more digestive enzymes. In some embodiments, the one or more digestive enzymes comprise one or more enzymes selected from the group consisting of proteases, amylases, celluloses, sucrases, maltases, papain, and lipases. In some embodiments, the one or more digestive enzymes comprise one or more pancreatic enzymes. The one or more digestive enzymes are, independently, derived from an animal source, a microbial source, a plant source, a fungal source, or are synthetically prepared. In some embodiments, the animal source is a pig pancreas.

In some embodiments, a pharmaceutical composition comprises at least one amylase, a mixture of proteases comprising chymotrypsin and trypsin, and at least one lipase. In some embodiments, a pharmaceutical composition comprises at least one protease and at least one lipase, and wherein the ratio of total proteases to total lipases (in USP units) ranges from about 1:1 to about 20:1.

In some embodiments, the pharmaceutical composition is a dosage formulation selected from the group consisting of: pills, tablets, capsules, caplets, sprinkles, creams, lotions, aerosols, emulsions, powders, liquids, gels, and a combination of any thereof.

In some embodiments, the pharmaceutical composition is formulated for oral administration, or for topical administration, or for intranasal, or for transmucosal administration.

Also provided is a method of treating a mammal or bird exhibiting one or more symptoms of an *E. coli* infection comprising administering to the mammal or bird a therapeutically effective amount of a composition comprising one or more digestive enzymes.

Further provided is a method for treating diarrhea comprising administering a pharmaceutical composition comprising one or more digestive enzymes to an individual. The disclosure also features a method for sanitizing or disinfecting a surface to reduce the amount of *E. coli* thereon or to eradicate the *E. coli* thereon, comprising applying to the surface a composition comprising one or more digestive enzymes. The surface can be a living surface (e.g., skin, wound) or an inanimate or non-living surface (e.g., medical device, food product).

Also provided herein is a method for reducing the amount of *E. coli* present on a skin region, tissue, or wound of a mammal or bird comprising applying to the skin region, tissue, or wound a composition comprising one or more digestive enzymes.

Also featured is a disinfectant comprising one or more digestive enzymes, wherein the disinfectant has a phenol coefficient of > 1 to about 20 for *S. aureus* or *E. coli*.

The disclosure also provides an antibiotic comprising one or more digestive enzymes, wherein the antibiotic is bacteriocidal and/or bacteriostatic for *E. coli*.

Similarly, a detergent comprising one or more digestive enzymes is also provided, wherein the detergent is bacteriocidal and/or bacteriostatic for *E. coli*.

Also provided is an antiseptic comprising one or more digestive enzymes, wherein the antiseptic is bacteriocidal and/or bacteriostatic for *E. coli*.

The disclosure also provides a disinfectant comprising one or more digestive enzymes, wherein the disinfectant is bacteriocidal and/or bacteriostatic for *E. coli*

.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Any reference disclosed herein, such as a patent, patent application, specification, book, article, or scientific publication, is incorporated by reference in its entirety. Other features, objects, and

advantages of the invention will be apparent from the description and drawings, and from the claims.

DETAILED DESCRIPTION

The term "administration" or "administering" refers to a method of giving a dosage of a composition or pharmaceutical composition to a vertebrate or invertebrate, including a mammal, a bird, a fish, or an amphibian, where the method is by any route, e.g., intrarespiratory, nasal, topical, oral, intravenous, intraperitoneal, intramuscular, transmucosal, buccal, rectal, vaginal, or sublingual. The preferred method of administration can vary depending on various factors, e.g., the components of the pharmaceutical composition, the site of the disease, the disease involved, and the severity of the disease.

The term "mammal" is used in its usual biological sense. Thus, it specifically includes humans, cattle, horses, dogs, and cats, but also includes many other species.

The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients, its use in the therapeutic compositions is contemplated. Supplementary active ingredients, such as antibiotics, antifungals, antimicrobials, can also be incorporated into the compositions. In addition, various adjuvants such as are commonly used in the art may be included. These and other such compounds are described in the literature, e.g., in the Merck Index, Merck & Company, Rahway, NJ. Considerations for the inclusion of various components in pharmaceutical compositions are described, e.g., in Gilman et al. (Eds.) (2006); Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 11th Ed., The McGraw-Hill Companies.

"Subject" or "patient" or "individual" as used herein, means a human or a non-human mammal, e.g., a dog, a cat, a mouse, a rat, a cow, a sheep, a pig, a goat, a non-human primate or a bird, e.g., a chicken, as well as any other vertebrate or invertebrate.

By "therapeutically effective amount" or "pharmaceutically effective amount" is typically one which is sufficient to achieve the desired effect and may vary according to the nature and severity of the disease condition, the nature of the subject, and the potency of the composition. It will be appreciated that different concentrations may be employed for prophylaxis than for treatment of an active disease. This amount can further depend upon the patient's height, weight, sex, age and medical history.

A therapeutic effect relieves, to some extent, one or more of the symptoms of the disease, and includes curing a disease. "Curing" means that the symptoms of active disease are eliminated. However, certain long-term or permanent effects of the disease may exist even after a cure is obtained (such as tissue damage).

"Treat," "treatment," or "treating," as used herein refers to administering a pharmaceutical composition for therapeutic purposes. The term "therapeutic treatment" refers to administering treatment to a patient already suffering from a disease thus causing a therapeutically beneficial effect, such as ameliorating existing symptoms, preventing additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms, postponing or preventing the further development of a disorder and/or reducing the severity of symptoms that will or are expected to develop.

The present disclosure provides compositions comprising one or more digestive enzymes and methods of using the same for the treatment and/or prevention of *E. coli* infections, including antibiotic-resistant forms of *E. coli* and virulent forms such as STEC, ETEC, or EPEC. The present disclosure also provides compositions comprising one or more digestive enzymes and methods of using the same as antiseptics, detergents, disinfectants, and sanitizers, e.g., as bacteriocidal and/or bacteriostatic compositions, to eradicate or attenuate the *E. coli* and/or to reduce its infectivity. The compositions described herein include one or more digestive enzymes, which are postulated to assist in the reduction, weakening, or eradication of *E. coli*, and thus to prevent contraction of *E. coli* infections or to treat *E. coli* infections (*e.g.,* improve or ameliorate the symptoms or reduce the time course of the infection).

Compositions

A composition for use as described herein can include one or more digestive enzymes. While not being bound by theory, it is believed that the digestive enzyme(s) in the composition can degrade *E. coli* cell wall, membrane, and/or protein structures, leading to the bacteriostatic and/or bacteriocidal activity. The compositions demonstrate species-specific bacteriocidal/bacteriostatic activity against *S. aureus* and *E. coli*, but not against *S. enterica*, possibly demonstrating that the vulnerability of the two organisms derives from proteolytic degradation of a similar protein sequence present in the two organisms.

A digestive enzyme as described herein is an enzyme that can break down one or more components of food (e.g., proteins, fats, carbohydrates). The digestive enzymes can be animal-derived (e.g., pancreatic or other digestive-track enzymes), or plant-, fungal-, or microorganism-derived enzymes, or can be synthetically prepared. Many digestive enzymes are commercially available or can be isolated and purified from other sources by methods well known to those having ordinary skill in the art. Enzymatic activity of the enzymes can also be evaluated using standard assays.

The digestive enzymes can be used in any combination of type of enzyme and any combination of enzyme sources. In some embodiments, the one or more digestive enzymes comprise one or more enzymes selected from the group consisting of proteases, amylases, celluloses, sucrases, maltases, papain (e.g., from papaya), bromelain (e.g., from pineapple), hydrolases, and lipases. In some embodiments, the one or more digestive enzymes comprise one or more pancreatic enzymes. In some embodiments, the composition comprises one or more proteases, one or more lipases, and one or more amylases. In some embodiments, the one or more proteases comprise chymotrypsin and trypsin. In some embodiments, a composition as described herein consists essentially of, or consists of, the one or more digestive enzymes.

In certain embodiments, the composition can comprise at least one amylase, at least two proteases, and at least one lipase. In certain embodiments, the composition can further include one or more hydrolases, papain, bromelain, papaya, celluloses, pancreatin, sucrases, and maltases.

As indicated, the one or more digestive enzymes can be derived from an animal source. In some embodiments, the animal source is a pig, e.g., a pig pancreas. Pig pancreatic enzyme extracts and formulations are known to those having ordinary skill in the art and are commercially available or can be prepared using known methods. For example, a pancreatic enzyme composition can be purchased from Scientific Protein Laboratories (designated PEC). A pancreatic enzyme composition, or any composition herein, can be adjusted to modify the amount of one or more digestive enzymes contained therein, e.g., the lipase, amylase, or protease content, such as by production and/or processing methods or by the selective addition of exogenous enzymes, activators, or inhibitors to the composition.

In certain circumstances, it may be desirable to have relatively higher activity of proteases than lipases. Thus, in some embodiments, a composition comprises at least one protease and at least one lipase, wherein the ratio of total proteases to total lipases (in USP units) ranges from about 1:1 to about 20:1 including 1:1, 2:1, 3;1, 4:1, 5;1, 6:1, 7:1, 8:1, 9:1, 10:1, 11;1, 12;1, 13;1, 14:1, 15:1, 16;1, 17:1, 18:1, 19:1 and 20:1, long with all values in-between. In some embodiments, the ratio of proteases to lipases ranges from about 4:1 to about 10:1 including 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, and 10:1, along with all values in-between.

In certain circumstances it may be useful to modify the amount of a particular enzymatic activity in a given composition. The activity of the one or more digestive enzymes can be adjusted in a variety of ways known to the skilled artisan, e.g., by increasing the amount of the particular enzyme, or by adjusting the components of the composition, e.g., via the use of stabilizers, inhibitors, and activators. In some embodiments, a composition described herein includes one or more proteases having an activity of from about 0.05 to about 400 USP Units per mg of the composition, or any value therebetween (e.g., 0.1; 0.2; 0.25; 0.5; 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 75, 100, 150, 200, 250, 300, 350 USP Units per mg). In some embodiments, a composition described herein includes one or more lipases having an activity of from about 0.005 to about 50 Units per mg of the composition, or any value therebetween (e.g., 0.01, 0.02, 0.025, 0.03, 0.04, 0.05, 0.06, 0.08, 0.1, 0.2, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 25, 28, 30, 35, 38, 40, 45 USP Units per mg). In some embodiments, a composition described herein includes one or more

amylases having an activity of from about 0.05 to about 400 USP Units per mg of the composition, or any value therebetween (e.g., 0.1; 0.2; 0.25; 0.5; 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 75, 100, 150, 200, 250, 300, 350 USP Units per mg). In some embodiments, a composition described herein includes one or more proteases in the above activity range, one or more lipases in the above activity range, and one or more amylases in the above activity range. One exemplary embodiment includes one or more proteases having an activity in the range of about 150-250 USP units/mg; one or more lipases having an activity in the range of about 20-40 USP units/mg; and one or more amylases having an activity in the range of about 200-300 USP units/mg.

In some embodiments, a composition can be formulated so as to stabilize the one or more digestive enzymes, e.g., to preserve the enzymatic activity of the enzymes. Stabilization techniques can limit or prevent auto-degradation of the one or more enzymes in a composition and help maintain enzymatic activity, increase shelf-life, and aid in the tolerance of the activity of the compositions to changes in temperature, humidity, and storage conditions. For example, in some embodiments, the one or more enzymes in the composition are encapsulated, e.g., lipid-encapsulated. In other applications, variations in excipients, pH, enzyme inhibitors, etc. can be employed to aid in stabilizing the enzymes. Appropriate stabilization techniques will depend on the intended application for the composition (e.g., antibiotic vs. detergent), the route of administration, the form of the composition, the intended site of delivery/activity, and other factors, and can be determined by those having ordinary skill in the art.

Certain useful enzyme activity stabilizers include compounds that provide a source of free calcium in a solution such as for example calcium salts; alkyl or branched alcohols such as for example ethanol and isopropyl alcohol; alkanolamines such as for example triethanolamine; acids, such as organic acids; and mixtures of petroleum distillates.

In certain embodiments, an enzyme activity stabilizer can be a composition selected from (1) compositions known to be effective in stabilizing enzymes in liquid aqueous solutions, including enzyme stabilizing compounds and systems, (2) selected "micelle inhibitors", and mixtures of (1) and (2). In some embodiments, the activity

stabilizer is a suitable concentration of boron anions. In some cases, the activity stabilizer is solvated in a polyol and may be combined with enzyme stabilizing synergists or adjuvants forming an enzyme stabilizing system. Preferred "micelle inhibitors" include species known to modify as well as to inhibit micelle formation and may be selected from water miscible solvents such as C1–C6 alkanols, C1–C6 diols, C2–C24 alkylene glycol ethers, alkylene glycol alkyl ethers, and mixtures thereof. A highly preferred micelle inhibitor is di-(propylene glycol) methyl ether ("DPM") and analogues thereof which modify micelle formation.

One example of an "enzyme stabilizing system" is a boron compound (e.g. boric acid) which in the past has been used alone or with selected other adjuvants and or synergists (e.g. polyfunctional amino compounds, antioxidants, etc) to protect proteolytic and other enzymes in storage and in various products.

An activity stabilizer may be chosen to substantially minimize the Minimum Inhibitory Concentration ("MIC") of digestive enzyme in the formulation. MIC is a measure of the minimum concentration of the biocide which succeeds in preventing bacterial growth in a culture during a specified time period, for example 24 hrs. Details of the MIC test are shown in "Bailey & Scott 'Diagnostic Microbiology', 8th edition, 1990 at page 177.

In some embodiments, a composition described herein can be coated with a variety or natural or synthetic coatings, e.g., to provide timed release of the enzymes, to provide flavor or odor masking, or to stabilize the enzymes. Coated enzyme preparations, including lipid-coated or lipid-encapsulated enzyme compositions, comprising one or more digestive enzymes useful for the methods and compositions described herein are disclosed in U.S. Ser. No. 12/386,051, filed April 13, 2009, incorporated herein by reference in its entirety. Such coated preparations can provide desired features, including increased shelf stability, reduced aerosolization of powder or solid formulations, odor and taste-masking, enzyme stabilization, and delayed or timed release of the enzymes.

Other additives for inclusion in the compositions described herein can be determined by those having ordinary skill in the art, and will be based on a number of features, including intended application, e.g., human vs. veterinary applications;

desired release profile; desired pharmacokinetics; safety; stability; and physical characteristics (smell, color, taste, pour, aerosilization). Suitable formulation ingredients, excipients, binders, bulking agents, flavorants, colorants, etc. can be determined and evaluated by methods known to those having ordinary skill.

Pharmaceutical Compositions and Antibiotics for Human or Veterinary Use

Compositions described herein can be formulated as pharmaceutical compositions, e.g., can include a composition as described previously formulated with one or more pharmaceutically acceptable carriers or excipients. The pharmaceutical compositions are useful for treating or preventing *E. coli* infections in humans and other animals, such as mammals (e.g., cows, horses, pigs, sheep, goat, monkey, cats, dogs, mice, rats) and birds (chickens, turkeys, ducks, geese). A pharmaceutical composition for treating *E. coli* infections can also be referred to as an antibiotic or antibiotic composition herein.

The susceptibility of *E. coli*, including virulent and resistant forms, to an antibiotic composition described herein can be determined by methods known to those having ordinary skill in the art. One rapid procedure uses commercially filter paper disks that have been impregnated with a specific quantity of the antibiotic composition. These disks are placed on the surface of agar plates that have been streaked with a culture of the *E. coli* being tested, and the plates are observed for zones of growth inhibition. The broth dilution susceptibility test involves preparing test tubes containing serial dilutions of the composition in liquid culture media, then inoculating the organism being tested into the tubes. The lowest concentration of drug that inhibits growth of the bacteria after a suitable period of incubation is reported as the minimum inhibitory concentration (MIC).

The resistance or susceptibility of *E. coli* to an antibiotic described herein can be determined on the basis of clinical outcome, *i.e.*, whether administration of that antibiotic to a subject infected by that organism will successfully cure the subject. Alternatively, to facilitate the identification of antibiotic resistance or susceptibility using *in vitro* test results, the National Committee for Clinical Laboratory Standards (NCCLS) has formulated standards for antibiotic susceptibility that correlate clinical

outcome to *in vitro* determinations of the minimum inhibitory concentration of antibiotic.

Administration of the pharmaceutical compositions herein can be via any of the accepted modes of administration for agents that serve similar utilities including, but not limited to, orally, subcutaneously, intravenously, intranasally, topically, transdermally, transmucosally, intraperitoneally, intramuscularly, intrapulmonarilly, vaginally, rectally, or intraocularly. Oral, transmucosal, topical, and parenteral administrations, for example, are customary in treating *E. coli* infection indications.

In the pharmaceutical compositions, effective concentrations of one or more digestive enzymes are mixed with a suitable pharmaceutical excipient or carrier. The concentrations of the digestive enzymes in the compositions are effective for delivery of an amount, upon administration, that is useful in the reduction or eradication of *E. coli* bacteria, and/or to treat or ameliorate of one or more of the symptoms associated with *E. coli* infection.

Antiobiotic compositions can be formulated for single dosage administration. To formulate a composition, the weight fraction of digestive enzymes is dissolved, suspended, dispersed or otherwise mixed in a selected carrier at an effective concentration such that the bacteria are reduced or eradicated, the treated condition is relieved, or one or more symptoms are ameliorated.

The digestive enzymes are included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the digestive enzymes in *in vitro* and *in vivo*, and then extrapolated therefrom for dosages for humans.

The concentration of digestive enzymes in the pharmaceutical composition will depend on absorption, inactivation and excretion rates of the enzymes, the physicochemical characteristics of the enzymes, the dosage schedule, the dosage form, and amount administered as well as other factors known to those of skill in the art.

The pharmaceutical composition may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing

protocols or by extrapolation from *in vivo* or *in vitro* test data. It is to be noted that concentrations and dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions.

Upon mixing or addition of the digestive enzymes, the resulting mixture may be a solution, suspension, gel, powder, emulsion or the like. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the digestive enzymes in the selected carrier or vehicle.

Compositions intended for pharmaceutical use may be administered as crystalline or amorphous products. Pharmaceutically acceptable compositions include solid, semi-solid, liquid, gel, powder, and aerosol dosage forms, such as, *e.g.*, tablets, capsules, caplets, sprinkles, powders, liquids, suspensions, emulsions, gels, suppositories, aerosols or the like. They may be obtained, for example, as films by methods such as precipitation, crystallization, freeze drying, spray drying, or evaporative drying. Microwave or radio frequency drying may be used for this purpose. The compositions can also be administered in sustained or controlled release dosage forms, including depot injections, osmotic pumps, pills, specialized coatings (e.g., enteric coatings) on oral dosage forms, transdermal (including electrotransport) patches, and the like, for prolonged and/or timed, pulsed administration at a predetermined rate. In some embodiments, the compositions are provided in unit dosage forms suitable for single administration of a precise dose.

The compositions can be administered either alone or more typically in combination with a conventional pharmaceutical carrier, excipient or the like. The term "excipient" is used herein to describe any ingredient other than the compound(s) (enzymes) used in the composition. Pharmaceutically acceptable excipients include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d-α-tocopherol polyethylene glycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as

Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium-chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethyl cellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, and wool fat. Cyclodextrins such as α-, β, and γ-cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-b-cyclodextrins, or other solubilized derivatives can also be advantageously used to enhance delivery of compositions described herein. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington: The Science and Practice of Pharmacy*, 21st Edition (Lippincott Williams & Wilkins. 2005).

In one preferred embodiment, the compositions will take the form of a unit dosage form such as a pill or tablet and thus the composition may contain, along with the active ingredient, a diluent such as lactose, sucrose, dicalcium phosphate, or the like; a lubricant such as magnesium stearate or the like; and a binder such as starch, gum acacia, polyvinylpyrrolidine, gelatin, cellulose, cellulose derivatives or the like. In another solid dosage form, a powder, solution or suspension (*e.g.*, in propylene carbonate, vegetable oils or triglycerides) is encapsulated in a gelatin capsule. Unit dosage forms in which two or more ingredients are physically separated are also contemplated; *e.g.*, capsules with granules of enzyme(s) and granules of other ingredients; two-layer tablets; two-compartment gel caps, etc.

Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc. one or more digestive enzymes and optional pharmaceutical adjuvants in a carrier (*e.g.*, water, saline, aqueous dextrose, glycerol, glycols, ethanol or the like) to form a solution or suspension. If desired, the pharmaceutical composition can also contain minor amounts of nontoxic auxiliary substances such as wetting agents, emulsifying agents, solubilizing agents, pH buffering agents and the like (*e.g.*, sodium acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine acetate, triethanolamine oleate, and the like). Injectables can be prepared in conventional forms, either as liquid solutions

or suspensions, as emulsions, or in solid forms suitable for dissolution or suspension in liquid prior to injection.

Solid compositions can be provided in various different types of dosage forms, depending on the physicochemical properties of the enzymes, the desired dissolution rate, cost considerations, and other criteria. In one of the embodiments, the solid composition is a single unit. This implies that one unit dose of the drug is comprised in a single, physically shaped solid form or article. In other words, the solid composition is coherent, which is in contrast to a multiple unit dosage form, in which the units are incoherent.

Examples of single units which may be used as dosage forms for the solid composition include tablets, such as compressed tablets, film-like units, foil-like units, wafers, lyophilized matrix units, and the like. In one embodiment, the solid composition is a highly porous lyophilized form. Such lyophilizates, sometimes also called wafers or lyophilized tablets, are particularly useful for their rapid disintegration, which also enables the rapid dissolution of the active compound.

On the other hand, for some applications the solid composition may also be formed as a multiple unit dosage form. Examples of multiple units are powders, sprinkles, granules, microparticles, microcapsules, pellets, beads, lyophilized powders, and the like. In one embodiment, the solid composition is a lyophilized powder. Such a dispersed lyophilized system comprises a multitude of powder particles, and due to the lyophilization process used in the formation of the powder, each particle has an irregular, porous microstructure through which the powder is capable of absorbing water very rapidly, resulting in quick dissolution. In another embodiment, the solid composition is a sprinkle formulation.

Another type of multiparticulate system which is also capable of achieving rapid drug dissolution is that of powders, granules, or pellets from water-soluble excipients which are coated with the composition ingredients (e.g., enzymes), so that the enzymes are located at the outer surface of the individual particles. In this type of system, the water-soluble low molecular weight excipient is useful for preparing the cores of such coated particles, which can be subsequently coated with a coating composition comprising the enzyme(s) and, preferably, one or more additional excipients, such as a binder, a pore former, a saccharide, a sugar alcohol, a film-

forming polymer, a plasticizer, or other excipients used in pharmaceutical coating compositions.

Appropriate dosages for treating or preventing *E. coli* infections will depend on the patient (species, age, weight, health), the severity of the disease, the strain of the *E. coli* present, the type of formulation (e.g., liquid or ointment) and other factors known to those having ordinary skill in the art. It is to be noted that concentrations and dosage values may vary with the severity of the condition to be alleviated. It is to be further understood that for any particular patient, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

In some embodiments, the pharmaceutical composition comprises per dose: amylases from about 10,000 to about 60,000 U.S.P, including 10,000, 15,000, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 55,000, and 60,000 U.S.P, along with all values in-between, proteases from about 10,000 to about 70,000 U.S.P, including 10,000, 15,000, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 55,000, 60,000, 65,000, and 70,000, along with all values in-between, and lipases from about 4,000 to about 30,000 U.S.P, including, 4,000, 5,000, 10,000, 15,000, 20,000, 25,000, and 30,000, along with all values in-between. A pharmaceutical composition can include one or more of : chymotrypsin from about 2 to about 5 mg including 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 mg, along with all values in-between; trypsin from about 60 to about 100 mg including 50, 65, 70, 75, 80, 85,90, 95, and 100 mg, including all values in between; papain from about 3,000 to about 10,000 USP units including 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, and 10,000 USP, along with all values in between; and papaya from about 30 to about 60 mg, including 30, 35, 40, 45, 50, 55, and 60 mg, along with all values in between.

Additional information on particular dosage forms of the compositions is provided below.

1. Compositions for oral administration

Oral pharmaceutical dosage forms are either solid, gel or liquid. The solid dosage forms are tablets, capsules, granules, and bulk powders. Types of oral tablets include compressed, chewable lozenges and tablets which may be enteric-coated, sugar-coated or film-coated. Capsules may be hard or soft gelatin capsules, while

granules and powders may be provided in non-effervescent or effervescent form with the combination of other ingredients known to those skilled in the art.

a. Solid compositions for oral administration

In certain embodiments, the formulations are solid dosage forms, in one embodiment, capsules or tablets. The tablets, pills, capsules, troches and the like can contain one or more of the following ingredients, or compounds of a similar nature: a binder; a lubricant; a diluent; a glidant; a disintegrating agent; a coloring agent; a sweetening agent; a flavoring agent; a wetting agent; an emetic coating; and a film coating. Examples of binders include microcrystalline cellulose, gum tragacanth, glucose solution, acacia mucilage, gelatin solution, molasses, polvinylpyrrolidine, povidone, crospovidones, sucrose and starch paste. Lubricants include talc, starch, magnesium or calcium stearate, lycopodium and stearic acid. Diluents include, for example, lactose, sucrose, starch, kaolin, salt, mannitol and dicalcium phosphate. Glidants include, but are not limited to, colloidal silicon dioxide. Disintegrating agents include crosscarmellose sodium, sodium starch glycolate, alginic acid, corn starch, potato starch, bentonite, methylcellulose, agar and carboxymethylcellulose. Coloring agents include, for example, any of the approved certified water soluble FD and C dyes, mixtures thereof; and water insoluble FD and C dyes suspended on alumina hydrate. Sweetening agents include sucrose, lactose, mannitol and artificial sweetening agents such as saccharin, and any number of spray dried flavors. Flavoring agents include natural flavors extracted from plants such as fruits and synthetic blends of compounds which produce a pleasant sensation, such as, but not limited to peppermint and methyl salicylate. Wetting agents include propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate and polyoxyethylene laural ether. Emetic-coatings include fatty acids, fats, waxes, shellac, ammoniated shellac and cellulose acetate phthalates. Film coatings include hydroxyethylcellulose, sodium carboxymethylcellulose, polyethylene glycol 4000 and cellulose acetate phthalate.

The digestive enzymes could be provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be formulated in an enteric coating that maintains its integrity in the stomach and releases the digestive enzymes in the intestine. The composition may also be formulated in combination with an antacid or other such ingredient.

When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents. The digestive enzymes can also be administered as a component of an elixir, suspension, syrup, wafer, sprinkle, chewing gum or the like. A syrup may contain, in addition to the active digestive enzymes, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The digestive enzymes can also be mixed with other active materials which do not impair the desired action, or with materials that supplement the desired action, such as antacids, H_2 blockers, and diuretics. Higher concentrations, up to about 98% by weight of the digestive enzymes may be included.

In all embodiments, tablets and capsules formulations may be coated as known by those of skill in the art in order to modify or sustain dissolution of the digestive enzymes. Thus, for example, they may be coated with a conventional enterically digestible coating, such as phenylsalicylate, waxes and cellulose acetate phthalate.

b. Liquid compositions for oral administration

Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules. Aqueous solutions include, for example, elixirs and syrups. Emulsions are either oil-in-water or water-in-oil.

Elixirs are clear, sweetened, hydroalcoholic preparations. Pharmaceutically acceptable carriers used in elixirs include solvents. Syrups are concentrated aqueous solutions of a sugar, for example, sucrose, and may contain a preservative. An emulsion is a two-phase system in which one liquid is dispersed in the form of small globules throughout another liquid. Pharmaceutically acceptable carriers used in emulsions are non-aqueous liquids, emulsifying agents and preservatives. Suspensions use pharmaceutically acceptable suspending agents and preservatives. Pharmaceutically acceptable substances used in non-effervescent granules, to be reconstituted into a liquid oral dosage form, include diluents, sweeteners and wetting agents. Pharmaceutically acceptable substances used in effervescent granules, to be reconstituted into a liquid oral dosage form, include organic acids and a source of

carbon dioxide. Coloring and flavoring agents are used in all of the above dosage forms.

Solvents include glycerin, sorbitol, ethyl alcohol and syrup. Examples of preservatives include glycerin, methyl and propylparaben, benzoic acid, sodium benzoate and alcohol. Examples of non-aqueous liquids utilized in emulsions include mineral oil and cottonseed oil. Examples of emulsifying agents include gelatin, acacia, tragacanth, bentonite, and surfactants such as polyoxyethylene sorbitan monooleate. Suspending agents include sodium carboxymethylcellulose, pectin, tragacanth, Veegum and acacia. Sweetening agents include sucrose, syrups, glycerin and artificial sweetening agents such as saccharin. Wetting agents include propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate and polyoxyethylene lauryl ether. Organic acids include citric and tartaric acid. Sources of carbon dioxide include sodium bicarbonate and sodium carbonate. Coloring agents include any of the approved certified water soluble FD and C dyes, and mixtures thereof. Flavoring agents include natural flavors extracted from plants such fruits, and synthetic blends of compounds which produce a pleasant taste sensation.

For a solid dosage form, the solution or suspension, in for example propylene carbonate, vegetable oils or triglycerides, is in one embodiment encapsulated in a gelatin capsule. Such solutions, and the preparation and encapsulation thereof, are disclosed in U.S. Patent Nos. 4,328,245; 4,409,239; and 4,410,545. For a liquid dosage form, the solution, e.g., for example, in a polyethylene glycol, may be diluted with a sufficient quantity of a pharmaceutically acceptable liquid carrier, e.g., water, to be easily measured for administration.

Alternatively, liquid or semi-solid oral formulations may be prepared by dissolving or dispersing the digestive enzymes in vegetable oils, glycols, triglycerides, propylene glycol esters (e.g., propylene carbonate) and other such carriers, and encapsulating these solutions or suspensions in hard or soft gelatin capsule shells. Other useful formulations include those set forth in U.S. Patent Nos. RE28,819 and 4,358,603. Briefly, such formulations include, but are not limited to, those containing digestive enzymes provided herein, a dialkylated mono- or poly-alkylene glycol, including, but not limited to, 1,2-dimethoxymethane, diglyme, triglyme, tetraglyme, polyethylene glycol-350-dimethyl ether, polyethylene glycol-550-dimethyl ether, polyethylene glycol-750-dimethyl ether wherein 350, 550 and 750 refer to the

approximate average molecular weight of the polyethylene glycol, and one or more antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, vitamin E, hydroquinone, hydroxycoumarins, ethanolamine, lecithin, cephalin, ascorbic acid, malic acid, sorbitol, phosphoric acid, thiodipropionic acid and its esters, and dithiocarbamates.

Other formulations include, but are not limited to, aqueous alcoholic solutions including a pharmaceutically acceptable acetal. Alcohols used in these formulations are any pharmaceutically acceptable water-miscible solvents having one or more hydroxyl groups, including, but not limited to, propylene glycol and ethanol. Acetals include, but are not limited to, di(lower alkyl) acetals of lower alkyl aldehydes such as acetaldehyde diethyl acetal.

2. Injectables, solutions and emulsions

Parenteral administration, in one embodiment characterized by injection, either subcutaneously, intramuscularly or intravenously is also contemplated herein. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. The injectables, solutions and emulsions also contain one or more excipients. Suitable excipients are, for example, water, saline, dextrose, glycerol or ethanol. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate and cyclodextrins.

Implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained (see, e.g., U.S. Patent No. 3,710,795) is also contemplated herein. Briefly, digestive enzymes provided herein are dispersed in a solid inner matrix, e.g., polymethylmethacrylate, polybutylmethacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethyleneterephthalate, natural rubber, polyisoprene, polyisobutylene, polybutadiene, polyethylene, ethylene-vinylacetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers such as hydrogels of esters of acrylic and methacrylic acid, collagen, cross-linked polyvinylalcohol and cross-linked partially hydrolyzed polyvinyl acetate, that is

surrounded by an outer polymeric membrane, e.g., polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/vinylacetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinylchloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinyloxyethanol copolymer, that is insoluble in body fluids. The digestive enzymes diffuse through the outer polymeric membrane in a release rate controlling step. The percentage of digestive enzymes contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the digestive enzyme or mixture thereof and the needs of the subject.

Parenteral administration of the compositions includes intravenous, subcutaneous and intramuscular administrations. Preparations for parenteral administration include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous.

If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances.

Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations must be added to parenteral preparations packaged in multiple-dose containers which include phenols or cresols, mercurials,

benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcelluose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate 80 (TWEEN® 80). A sequestering or chelating agent of metal ions include EDTA. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles; and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

The unit-dose parenteral preparations are packaged in an ampoule, a vial or a syringe with a needle. All preparations for parenteral administration must be sterile, as is known and practiced in the art.

Illustratively, intravenous or intraarterial infusion of a sterile aqueous solution containing digestive enzymes is an effective mode of administration. Another embodiment is a sterile aqueous or oily solution or suspension containing digestive enzymes that can be injected as necessary to produce the desired pharmacological effect.

Injectables are designed for local and systemic administration. In one embodiment, a therapeutically effective dosage is formulated to contain a concentration of at least about 0.1% w/w up to about 90% w/w or more, in certain embodiments more than 1% w/w of the digestive enzymes to the treated tissue(s).

The digestive enzymes may be suspended in micronized or other suitable form or may be derivatized to produce a more soluble active product. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the digestive enzymes in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the condition and may be empirically determined.

3. Lyophilized powders

Of interest herein are also lyophilized powders, which can be reconstituted for administration as solutions, emulsions and other mixtures. They may also be reconstituted and formulated as solids or gels.

The sterile, lyophilized powder is prepared by dissolving digestive enzymes as provided herein in a suitable solvent. The solvent may contain an excipient which improves the stability or other pharmacological component of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, dextrose, sorbital, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may also contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides the desired formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial will contain a single dosage or multiple dosages of the digestive enzymes. The lyophilized powder can be stored under appropriate conditions, such as at about 4 °C to room temperature.

Reconstitution of this lyophilized powder with water for injection provides a formulation for use in parenteral administration. For reconstitution, the lyophilized powder is added to sterile water or other suitable carrier. The precise amount depends upon the selected digestive enzymes. Such amount can be empirically determined.

4. Topical administration

Topical mixtures can be prepared as described for local and systemic administration. The resulting mixture may be a solution, suspension, emulsions or the like and are formulated as creams, gels, ointments, emulsions, powders, solutions, elixirs, lotions, suspensions, tinctures, pastes, foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other formulations suitable for topical administration.

The digestive enzymes may be formulated as aerosols for topical application, such as by inhalation (see, e.g., U.S. Patent Nos. 4,044,126, 4,414,209, and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment of inflammatory diseases, particularly asthma). These formulations for administration to the respiratory tract can be in the form of an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case, the particles of the formulation will, in one embodiment, have diameters of less than 50 microns, in one embodiment less than 10 microns.

The digestive enzymes may be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Topical administration is contemplated for transdermal delivery and also for administration to the eyes or mucosa, or for inhalation therapies. Nasal solutions of the digestive enzymes alone or in combination with other pharmaceutically acceptable excipients can also be administered.

These solutions, particularly those intended for ophthalmic use, may be formulated as 0.01% - 10% isotonic solutions, pH about 5-7, with appropriate salts.

Powders can be formed with the aid of any suitable powder base, e.g., talc, lactose, starch, and the like. Solutions can be formulated with an aqueous or non-aqueous base, and can also include one or more dispersing agents, suspending agents, solubilizing agents, and the like. Topical gels are prepared using polymers having a molecular weight and level of concentration effective to form a viscous solution or colloidal gel of an aqueous or non-aqueous solution or suspension of digestive enzymes. Polymers from which topical gels may be prepared include polyphosphoesters, polyethylene glycols, high molecular weight poly(lactic) acids, hydroxypropyl celluloses, chitosan, polystyrene sulfonates, and the like.

Ointments, creams and lotions are formulated, for example, with an aqueous or oily base and addition of a suitable thickening agent, gelling agent, stabilizing agent, emulsifying agent, dispersing agent, suspending agent, or consistency regulating agent, and the like. Bases include water, an alcohol or an oil, such as liquid paraffin, mineral oil, or a vegetable oil, such as peanut or castor oil. Thickening agents that can be used according to the nature of the base include soft paraffin, aluminum stearate, cetostearyl alcohol, propylene glycol, polyethylene glycols, polyphosphoesters, poly(lactic acids), hydroxyethyl celluloses, hydroxypropyl celluloses, cellulose gums, acrylate polymers, hydrophilic gelling agents, chitosan, polystyrene sulfonate, petrolatum, woolfat, hydrogenated lanolin, beeswax, and the like.

The ointments, pastes, creams, gels, and lotions can also contain excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc, zinc oxide,

and mixtures thereof. Powders and sprays can also contain excipients such as silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Solutions, suspensions or dispersions can be converted into aerosols or sprays by any of the known means routinely used for making aerosols for topical application. In general, such methods comprise pressurizing or providing a means of pressurizing a container of a solution, suspension or dispersion, usually with an inert carrier gas, and passing the pressured gas through a small orifice. Sprays and aerosols can also contain customary propellants, e.g., chlorofluorohydrocarbons or volatile unsubstituted hydrocarbons, such as butane and propane.

Excipients include compounds that promote skin absorption, such as dimethyl sulfoxide (DMSO), partial glycerides of fatty acids, and the like, present at levels up to about 10 wt % of the total formula weight. Examples of partial fatty acid glycerides include, but are not limited to IMWITOR 742 and IMWITOR 308 available from SASOL North America, Inc. of Houston, Tex. The topical formulations may also optionally include inactive ingredients to improve cosmetic acceptability, including but not limited to, humectants, surfactants, fragrances, coloring agents, emollients, fillers, and the like.

The topical compositions may also include other antibiotic agents, examples of which include bacitracin, neomycin, polymixin, beta-lactams, including penicillin, methicillin, moxalactam and cephalosporins, such as cefaclor, cefadroxil, cefamandole nafate, cefazolin, cefixime, cefinetazole, cefonioid, cefoperazone, ceforanide, cefotanme, cefotaxime, cefotetan, cefoxitin, cefpodoxime proxetil, ceftazidime, ceftizoxime, ceftriaxone, cefriaxone, cefuroxime, cephalexin, cephalosporin C, cepahlosporin C sodium salt, cephalothin, cephalothin sodium salt, cephalothin dihydrate, cephapirin, cephradine, cefuroximeaxetil, loracarbef, and the like. Essentially any anti-infective/antibiotic agent that is effective when applied topically can be used. Thus, the methods of the present invention for both treating active infections and decolonizing skin pathogen populations include methods in which digestive enzymes are applied singularly or in combination, either with no other anti-infective agent, or with at least one other anti-infective agent.

The topical compositions can be administered directly by the dusting of a powder, spraying of an aerosol or by spreading a film of an ointment, cream, lotion, solution or gel to the desired area of the skin using the fingertips of the patient or a

healthcare provider or other conventional application such as a swab or wipe. The product may be first applied to the skin and spread with the fingertips or an applicator or applied to the fingertips and spread over the skin. The compositions may also optionally first be coated on the surface of a topical applicator, such as a bandage, swab, moist woven or non-woven wipe and the like, which is then applied to the portion of the skin to receive the composition.

The topical compositions of the present invention can be prepared with base formulations that are essentially conventional to one of ordinary skill in the art with respect to the ingredients employed, quantities thereof, and methods of preparation, all of which require no further description. Topical compositions according to the present invention can also be prepared as a cream or lotion based on an emulsion formulation possessing heretofore unrecognized bactericidal activity, in addition to good skin compatibility and wound-healing properties that is particularly well-suited for formulation with digestive enzymes.

As discussed above, the present invention is not limited to topical cream or lotion formulations. Topical formulations based on conventional sprays, mists, aerosols, lotions, creams, aqueous and non-aqueous solutions or liquids, oils, gels, ointments, pastes, unguents, emulsions and suspensions will contain an amount of digestive enzymes, and optionally one or more other anti-infective agents, in a total concentration of between about 0.125 and about 10% by weight or more, recognizing again that optimal dosages may differ only by 0.05% by weight, so that representative cream and lotion embodiments will include every 0.05% by weight concentration increment within this range.

The topical compositions of the present invention are used to treat skin infections and wound infections such as surface wounds and penetrating wounds. Wounds suitable for treatment include wounds in skin abrasions, skin or surface cuts, decubiti, burns and surgical wounds. The topical compositions of the present invention can be used as well to decolonize populations of *E. coli* bacteria to prevent secondary, including the pre-treatment of areas prior to surgery or catheter insertion.

Mucosal delivery formulations can include digestive enzymes as described herein combined or coordinately administered with a suitable carrier or vehicle for mucosal delivery. As used herein, the term "carrier" means a pharmaceutically acceptable solid or liquid filler, diluent or encapsulating material. A water-containing

liquid carrier can contain pharmaceutically acceptable additives such as acidifying agents, alkalizing agents, antimicrobial preservatives, antioxidants, buffering agents, chelating agents, complexing agents, solubilizing agents, humectants, solvents, suspending and/or viscosity-increasing agents, tonicity agents, wetting agents or other biocompatible materials. A tabulation of ingredients listed by the above categories, can be found in the U.S. Pharmacopeia National Formulary, pp. 1857-1859, 1990. Some examples of the materials which can serve as pharmaceutically acceptable carriers are sugars, such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols such as glycerin, sorbitol, mannitol and polyethylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen free water; isotonic saline; Ringer's solution, ethyl alcohol and phosphate buffer solutions, as well as other non toxic compatible substances used in pharmaceutical formulations. Wetting agents, emulsifiers and lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions, according to the desires of the formulator. Examples of phannaceutically acceptable antioxidants include water soluble antioxidants such as ascorbic acid, cysteine hydrochloride, sodium bisulfite, sodium metabisulfite, sodium sulfite and the like; oil- soluble antioxidants such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol and the like; and metal-chelating agents such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid and the like. The amount of digestive enzymes that can be combined with the carrier materials to produce a single dosage form will vary depending upon the particular mode of administration.

Mucosal formulations are generally sterile, particulate free and stable for pharmaceutical use. As used herein, the term "particulate free" means a formulation that meets the requirements of the USP specification for small volume parenteral

solutions. The term "stable" means a formulation that fulfills all chemical and physical specifications with respect to identity, strength, quality, and purity that have been established according to the principles of Good Manufacturing Practice, as set forth by appropriate governmental regulatory bodies.

Within the mucosal delivery compositions, various delivery-enhancing agents can be employed which enhance delivery of digestive enzymes into or across a mucosal surface. As used herein, "mucosal delivery-enhancing agents" include agents which enhance the release or solubility (e.g., from a formulation delivery vehicle), diffusion rate, penetration capacity and timing, uptake, residence time, stability, effective half-life, peak or sustained concentration levels, clearance and other desired mucosal delivery characteristics (e.g., as measured at the site of delivery, or at a selected target site of activity such as the bloodstream or central nervous system) of digestive enzymes or other biologically active compound(s). Enhancement of mucosal delivery can thus occur by any of a variety of mechanisms, for example by increasing the diffusion, transport, persistence or stability of the digestive enzymes, increasing membrane fluidity, modulating the availability or action of calcium and other ions that regulate intracellular or paracellular permeation, solubilizing mucosal membrane components (e.g., lipids), changing non-protein and protein sulfhydryl levels in mucosal tissues, increasing water flux across the mucosal surface, modulating epithelial junctional physiology, reducing the viscosity of mucus overlying the mucosal epithelium, reducing mucociliary clearance rates, and other mechanisms.

While the mechanism of absorption promotion may vary with different intranasal delivery-enhancing agents of the invention, useful reagents in this context will not substantially adversely affect the mucosal tissue and will be selected according to the physicochemical characteristics of the particular digestive enzymes or other active or delivery- enhancing agent. In this context, delivery-enhancing agents that increase penetration or permeability of mucosal tissues will often result in some alteration of the protective permeability barrier of the mucosa. For such delivery-enhancing agents to be of value within the invention, it is generally desired that any significant changes in permeability of the mucosa be reversible within a time frame appropriate to the desired duration of drug delivery. Furthermore, there should be no substantial, cumulative toxicity, nor any permanent deleterious changes induced in the barrier properties of the mucosa with long- term use.

In some embodiments, absorption-promoting agents for coordinate administration or combinatorial formulation with the digestive enzymes as described herein are selected from small hydrophilic molecules, including but not limited to, dimethyl sulfoxide (DMSO), dimethylformamide, ethanol, propylene glycol, and the 2-pyrrolidones. Alternatively, long-chain amphipathic molecules, for example, deacylmethyl sulfoxide, azone, sodium lauryl sulfate, oleic acid, and the bile salts, may be employed to enhance mucosal penetration of the digestive enzymes. In additional aspects, surfactants (e.g., polysorbates) are employed as adjunct compounds, processing agents, or formulation additives to enhance intranasal delivery of the digestive enzymes. These penetration-enhancing agents typically interact at either the polar head groups or the hydrophilic tail regions of molecules that comprise the lipid bilayer of epithelial cells lining the nasal mucosa (Barry, Pharmacology of the Skin, Vol. 1, pp. 121-137, Shroot et al., Eds., Karger, Basel, 1987; and Barry, J. controlled Release 6:85-97, 1987). Interaction at these sites may have the effect of disrupting the packing of the lipid molecules, increasing the fluidity of the bilayer, and facilitating transport of the digestive enzymes across the mucosal barrier. Interaction of these penetration enhancers with the polar head groups may also cause or permit the hydrophilic regions of adjacent bilayers to take up more water and move apart, thus opening the paracellular pathway to transport of the digestive enzymes. In addition to these effects, certain enhancers may have direct effects on the bulk properties of the aqueous regions of the nasal mucosa. Agents such as DMSO, polyethylene glycol, and ethanol can, if present in sufficiently high concentrations in delivery environment (e.g., by pre-administration or incorporation in a therapeutic formulation), enter the aqueous phase of the mucosa and alter its solubilizing properties, thereby enhancing the partitioning of the digestive enzymes from the vehicle into the mucosa.

Additional mucosal delivery-enhancing agents that are useful within the coordinate administration and processing methods and combinatorial formulations of the invention include, but are not limited to, mixed micelles; enamines; nitric oxide donors (e.g., S- nitroso-N-acetyl-DL-penicillamine, NOR1, NOR4, which are preferably co-administered with an NO scavenger such as carboxy-PITO or doclofenac sodium); sodium salicylate; glycerol esters of acetoacetic acid (e.g., glyceryl- 1, 3 -diacetoacetate or 1,2- isopropylideneglycerine-3-acetoacetate); and

other release-diffusion or intra- or trans- epithelial penetration-promoting agents that are physiologically compatible for mucosal delivery. Other absorption-promoting agents are selected from a variety of carriers, bases and excipients that enhance mucosal delivery, stability, activity or trans-epithelial penetration of the digestive enzymes. These include, inter alia, clyclodextrins and β-cyclodextrin derivatives (e.g., 2-hydroxypropyl-β-cyclodextrin and heptakis(2,6-di-O-methyl-β- cyclodextrin). These compounds, optionally conjugated with one or more of the active ingredients and further optionally formulated in an oleaginous base, enhance bioavailability in the mucosal formulations of the invention. Yet additional absorption-enhancing agents adapted for mucosal delivery include medium-chain fatty acids, including mono- and diglycerides (e.g., sodium caprate-extracts of coconut oil, Capmul), and triglycerides (e.g., amylodextrin, Estaram 299, Miglyol 810).

The mucosal therapeutic and prophylactic compositions may be supplemented with any suitable penetration-promoting agent that facilitates absorption, diffusion, or penetration of digestive enzymes across mucosal barriers. The penetration promoter may be any promoter that is pharmaceutically acceptable. Thus, in more detailed aspects of the invention compositions are provided that incorporate one or more penetration-promoting agents selected from sodium salicylate and salicylic acid derivatives (e.g., acetyl salicylate, choline salicylate, salicylamide); amino acids and salts thereof (e.g. monoaminocarboxlic acids such as glycine, alanine, phenylalanine, proline, hydroxyproline; hydroxyamino acids such as serine; acidic amino acids such as aspartic acid, glutamic acid; and basic amino acids such as lysine— inclusive of their alkali metal or alkaline earth metal salts); and N-acetylamino acids (N-acetylalanine, N-acetylphenylalanine, N-acetylserine, N-acetylglycine, N-acetyllysine, N-acetylglutamic acid, N-acetylproline, N-acetylhydroxyproline, etc.) and their salts (alkali metal salts and alkaline earth metal salts). Also provided as penetration-promoting agents within the methods and compositions of the invention are substances which are generally used as emulsifiers (e.g. sodium oleyl phosphate, sodium lauryl phosphate, sodium lauryl sulfate, sodium myristyl sulfate, polyoxyethylene alkyl ethers, polyoxyethylene alkyl esters, etc.), caproic acid, lactic acid, malic acid and citric acid and alkali metal salts thereof, pyrrolidonecarboxylic acids, alkylpyrrolidonecarboxylic acid esters, N-alkylpyrrolidones, proline acyl esters, and the like.

5. Compositions for other routes of administration

Other routes of administration, such as transdermal patches, including iontophoretic and electrophoretic devices, and rectal administration, are also contemplated herein.

Transdermal patches, including iotophoretic and electrophoretic devices, are well known to those of skill in the art. For example, such patches are disclosed in U.S. Patent Nos. 6,267,983, 6,261,595, 6,256,533, 6,167,301, 6,024,975, 6,010715, 5,985,317, 5,983,134, 5,948,433, and 5,860,957.

For example, pharmaceutical dosage forms for rectal administration are rectal suppositories, capsules and tablets for systemic effect. Rectal suppositories are used herein mean solid bodies for insertion into the rectum which melt or soften at body temperature releasing one or more pharmacologically or therapeutically active ingredients. Pharmaceutically acceptable substances utilized in rectal suppositories are bases or vehicles and agents to raise the melting point. Examples of bases include cocoa butter (theobroma oil), glycerin-gelatin, carbowax (polyoxyethylene glycol) and appropriate mixtures of mono-, di- and triglycerides of fatty acids. Combinations of the various bases may be used. Agents to raise the melting point of suppositories include spermaceti and wax. Rectal suppositories may be prepared either by the compressed method or by molding. The weight of a rectal suppository, in one embodiment, is about 2 to 3 gm.

Tablets and capsules for rectal administration are manufactured using the same pharmaceutically acceptable substance and by the same methods as for formulations for oral administration.

6. Sustained Release Formulations

Also provided are sustained release formulations to deliver the digestive enzymes to the desired target. It is understood that the digestive enzymes levels are maintained over a certain period of time as is desired and can be easily determined by one skilled in the art. Such sustained and/or timed release formulations may be made by sustained release means of delivery devices that are well known to those of ordinary skill in the art, such as those described in US Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3, 598,123; 4,008,719; 4,710,384; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556 and 5,733,566, the disclosures

of which are each incorporated herein by reference. These pharmaceutical compositions can be used to provide slow or sustained release of one or more digestive enzymes using, for example, hydroxypropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or the like. Suitable sustained release formulations known to those skilled in the art, including those described herein, may be readily selected for use with the pharmaceutical compositions provided herein. Thus, single unit dosage forms suitable for oral administration, such as, but not limited to, tablets, capsules, gelcaps, caplets, powders and the like, that are adapted for sustained release are contemplated herein.

In one embodiment, the sustained release formulation contains an active compound such as, but not limited to, microcrystalline cellulose, maltodextrin, ethylcellulose, and magnesium stearate. As described above, all known methods for encapsulation which are compatible with properties of the disclosed digestive enzymes are contemplated herein. The sustained release formulation is encapsulated by coating particles or granules of the pharmaceutical compositions provided herein with varying thickness of slowly soluble polymers or by microencapsulation. In one embodiment, the sustained release formulation is encapsulated with a coating material of varying thickness (e.g. about 1 micron to 200 microns) that allow the dissolution of the pharmaceutical composition about 48 hours to about 72 hours after administration to a mammal. In another embodiment, the coating material is a food-approved additive.

In another embodiment, the sustained release formulation is a matrix dissolution device that is prepared by compressing the drug with a slowly soluble polymer carrier into a tablet. In one embodiment, the coated particles have a size range between about 0.1 to about 300 microns, as disclosed in U.S. Patent Nos. 4,710,384 and 5,354,556, which are incorporated herein by reference in their entireties. Each of the particles is in the form of a micromatrix, with the active ingredient uniformly distributed throughout the polymer.

The digestive enzymes provided herein can be formulated as a sustained and/or timed release formulation. All sustained release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-sustained counterparts. Ideally, the use of an optimally designed sustained release preparation in

medical treatment is characterized by a minimum of digestive enzymes being employed to cure or control the condition. Advantages of sustained release formulations may include: 1) extended activity of the composition, 2) reduced dosage frequency, and 3) increased patient compliance. In addition, sustained release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the composition, and thus can affect the occurrence of side effects.

The sustained release formulations provided herein are designed to initially release an amount of the therapeutic composition that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of compositions to maintain this level of therapeutic effect over an extended period of time. In order to maintain this constant level in the body, the therapeutic composition must be released from the dosage form at a rate that will replace the composition being metabolized and excreted from the body.

The sustained release of an active ingredient may be stimulated by various inducers, for example pH, temperature, enzymes, water, or other physiological conditions or compounds.

Preparations for oral administration may be suitably formulated to give controlled release of the digestive enzymes. In one embodiment, the digestive enzymes are formulated as controlled release powders of discrete microparticles that can be readily formulated in liquid form. The sustained release powder comprises particles containing an active ingredient and optionally, an excipient with at least one non-toxic polymer.

The powder can be dispersed or suspended in a liquid vehicle and will maintain its sustained release characteristics for a useful period of time. These dispersions or suspensions have both chemical stability and stability in terms of dissolution rate. The powder may contain an excipient comprising a polymer, which may be soluble, insoluble, permeable, impermeable, or biodegradable. The polymers may be polymers or copolymers. The polymer may be a natural or synthetic polymer. Natural polymers include polypeptides (e.g., zein), polysaccharides (e.g., cellulose), and alginic acid. Representative synthetic polymers include those described, but not limited to, those described in column 3, lines 33-45 of U.S. Patent No. 5,354,556, which is incorporated by reference in its entirety. Particularly suitable polymers

include those described, but not limited to those described in column 3, line 46-column 4, line 8 of U.S. Patent No. 5,354,556 which is incorporated by reference in its entirety.

The sustained release compositions provided herein may be formulated for parenteral administration, e.g., by intramuscular injections or implants for subcutaneous tissues and various body cavities and transdermal devices. In one embodiment, intramuscular injections are formulated as aqueous or oil suspensions. In an aqueous suspension, the sustained release effect is due to, in part, a reduction in solubility of the digestive enzymes upon complexation or a decrease in dissolution rate. A similar approach is taken with oil suspensions and solutions, wherein the release rate of digestive enzymes is determined by partitioning of the digestive enzymes out of the oil into the surrounding aqueous medium. Only digestive enzymes which are oil soluble and have the desired partition characteristics are suitable. Oils that may be used for intramuscular injection include, but are not limited to, sesame, olive, arachis, maize, almond, soybean, cottonseed and castor oil.

Coadministration with other Pharmaceutical Compositions

The pharmaceutical compositions can be used on their own, and/or in combination with other therapeutic or antibiotic (e.g., *anti-E. coli*) regimens. For example, a patient can be administered other therapeutic agents, such as anti-inflammatories or anesthetics, to address other aspects of an *E. coli* infection (e.g., pain, tissue damage) or still other illnesses that the patient may be facing. In other embodiments, a patient can be administered a pharmaceutical composition as described herein, and one or more additional antibiotics. The one or more additional antibiotics can be effective against *E. coli* or other bacteria, or both (e.g., if the patient has multiple infections) and can be in the same or a different format from the present pharmaceutical compositions (e.g., one can be a liquid and one can be a topical antibiotic). The major classes of antibiotics are (1) the β-lactams, including the penicillins, cephalosporins and monobactams; (2) the aminoglycosides, e.g., gentamicin, tobramycin, netilmycin, and amikacin; (3) the tetracyclines; (4) the sulfonamides and trimethoprim; (5) the fluoroquinolones, e.g., ciprofloxacin, norfloxacin, and ofloxacin; (6) vancomycin; (7) the macrolides, which include for

example, erythromycin, azithromycin, and clarithromycin; and (8) other antibiotics, e.g., the polymyxins, chloramphenicol and the lincosamides.

In some embodiments, the additional antibiotic can be a beta-lactam antibiotic (e.g., a penicillin or penicillin derivatives, a cephalosporin, a monobactam, a penam, a penem, an oxapenam, a carbapenem or a cabapenam, a cephem, a carbacephem, an oxacephem, a monobactam). In some embodiments, the additional antibiotic can be a beta-lactamase inhibitor. In some embodiments, the additional antibiotic can be an aminoglycosidic antibiotic. In some embodiments, the additional antibiotic is selected from penicillin or a penicillin derivative, oxacillin, amoxicillin, nafcillin, cloxacillin, methicillin, temocillin, ampicillin, co-amoxiclav, azlocillin, carbenicillin, ticarcillin, mezlocillin, piperacillin, cephalexin, cephalothin, cefazolin, cefaclor, cefuroxime, cefamandole, cefotetan, cefoxitin, ceftriaxone, cefotaxime, cefpodoxime, ceftazidime, cefepime, cefpirome, vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, decaplanin, oritavancin, and dalbavancin.

An antibiotic composition described herein and an additional antibiotic can be administered separately or in a single dosage form. If separately, they can be administered in any order and relative frequency.

Disinfectants and Sanitizers

Compositions comprising one or more digestive enzymes as described herein can also be used as disinfectants and sanitizers, *e.g.,* to disinfect inanimate objects and surfaces in, without limitation, hospital, health care, home, and community settings by eradicating, attenuating or reducing *E. coli* in such locations. Disinfectants are antimicrobial agents that are applied to non-living objects to destroy microorganisms. Disinfectants should generally be distinguished from antibiotics that destroy microorganisms within the body, and from antiseptics, which destroy microorganisms on living tissue. Sanitizers are disinfectants that reduce the number of microorganisms to a safe level. One definition of a sanitizer states that a sanitizer must be capable of killing 99.999%, known as a 5 log reduction, of a specific bacterial test population, and to do so within 30 seconds. The main difference between a sanitizer and a disinfectant is that at a specified use dilution, the disinfectant must have a higher kill capability for pathogenic bacteria compared to that of a sanitizer.

A disinfectant or sanitizer as described herein can include one or more digestive enzymes, in various embodiments as described previously above, and can optionally include other active and inactive ingredients, including stabilizers (e.g., enzyme stabilizers), other disinfectants known to those having ordinary skill in the art, formulation excipients, colorants, perfumes, etc. One having ordinary skill in the art can select the additional active or inactive ingredients to include in a disinfectant. Examples of additional disinfectants include: sources of active chlorine (i.e., hypochlorites, chloramines, dichloroisocyanurate and trichloroisocyanurate, wet chlorine, chlorine dioxide etc.); sources of active oxygen (peroxides, such as peracetic acid, potassium persulfate, sodium perborate, sodium percarbonate and urea perhydrate); iodine and iodophor solutions (iodpovidone (povidone-iodine, Betadine), Lugol's solution, iodine tincture, iodinated nonionic surfactants); concentrated alcohols (mainly ethanol, 1-propanol, called also n-propanol and 2-propanol, called isopropanol and mixtures thereof; further, 2-phenoxyethanol and 1- and 2-phenoxypropanols); phenolic substances (such as phenol (also called "carbolic acid"), cresols (called "Lysole" in combination with liquid potassium soaps), halogenated (chlorinated, brominated) phenols, such as hexachlorophene, triclosan, trichlorophenol, tribromophenol, pentachlorophenol, Dibromol and salts thereof); cationic surfactants, such as some quaternary ammonium cations (such as benzalkonium chloride, cetyl trimethylammonium bromide or chloride, didecyldimethylammonium chloride, cetylpyridinium chloride, benzethonium chloride) and others; non-quarternary compounds, such as chlorhexidine, glucoprotamine, octenidine dihydrochloride; strong oxidizers, such as ozone and permanganate solutions; heavy metals and their salts, such as colloidal silver, silver nitrate, mercury chloride, phenylmercury salts, copper sulfate, and copper oxide-chloride; concentrated strong acids (phosphoric, nitric, sulfuric, amidosulfuric, toluenesulfonic acids) and alkalis (sodium, potassium, calcium hydroxides), such as of pH < 1 or > 13, particularly under elevated temperature (above 60°C). In some cases, a disinfectant as described herein will consist essentially of the one or more digestive enzymes. In some cases, a disinfectant will consist essentially of the one or more digestive enzymes, and will not include additional disinfecting agents.

A disinfectant composition comprising one or more digestive enzymes as described herein can be incorporated with other ingredients to form a variety of

disinfectant products including but not limited to hand cleansers, mouthwashes, surgical scrubs, body splashes, hand sanitizer gels and foams, disinfectant wipes, and similar personal care products. Additional types of products include disinfectant foams, creams, mousses, and the like, and compositions containing organic and inorganic filler materials, such as emulsions, lotions, creams, pastes, and the like. The compositions can also be used as an antibacterial cleanser for hard surfaces, for example, sinks and countertops in hospitals, food service areas, and meat processing plants. The disinfectant compositions can also be used as disinfectant fogs and disinfectant mists. The present digestive enzyme compositions can be manufactured as dilute ready-to-use compositions, or as concentrates that are diluted prior to use. The various products in which the disinfectants are used may also include fragrances, depending on the nature of the product. For example, a pine or lemon fragrance may be desirable for use for kitchen cleaning wipes because of their appealing association with cleanliness to many consumers. Further, gels or aerosols may also be fragranced for similar or other reasons.

In one embodiment, the disinfectant compositions can be used to make disinfectant wipes. A disinfectant wipe can be used to clean a variety of hard and other surfaces, including, for example, human hands and skin, medical instruments and devices, countertops, floors, walls, and windows. Wipes can be made of a variety of fabrics. Fabrics are defined to include cloths and papers, as well as woven and non-woven materials. The woven or nonwoven fabrics can be made of suitable materials such as rayon, nylon, or cotton, and combinations thereof. Examples of nonwoven fabrics are described in U.S. Pat. Nos. 3,786,615; 4,395,454; and 4,199,322; which are hereby incorporated by reference. The fabrics or papers can be impregnated with the disinfectant solution by any method known in the art. The wipes can be packaged in any manner known in the art including individual blister-packs or wrapped or stacked multi-packs.

In another embodiment, the disinfectant composition comprising one or more digestive enzymes can be formulated into a gel or gelatinous sanitization composition. In addition to the disinfectant compositions, the gel sanitizers can include a thickening or gelling agent, wherein "thickening agent" and "gelling agent" are used interchangeably. As used herein, the terms "gel" or "gelatinous" sanitization

compositions refers to a disinfectant liquid substances that can have a viscosity from about 1,000 centipoise to about 100,000 centipoise, or from 2,000 centipoise to 50,000 centipoise in another embodiment, though these ranges are not intended to be limiting. For example, a hand gel may be considerably less viscous than a gel used for industrial cleaning or disinfectant purposes. Examples of gelling or thickening agents include but are not limited to natural gum such as guar and guar derivatives, a synthetic polymer, a clay, an oil, a wax, aloe vera gel, an acrylate homopolymer, an acrylate copolymer, a carbomer, cellulose, a cellulose derivative, algin, an algin derivative, a water-insoluble C8-C20 alcohol, carrageenan, fumed silica, mixtures thereof, and the like. The gelling agent can be present in the gelatinous sanitation composition in an amount from about 0.1 wt % to 50 wt % of the gelatinous composition. In another embodiment, the gelling agent is present in an amount from 0.25 wt % to 10 wt % of the gelatinous composition. The amount of gelling agent can be dependent on a variety of factors including the type of gelling agent and the desired viscosity of the gel. The gelatinous sanitizers can be used for a variety of applications including sanitization of human skin e.g., gel hand sanitizer, and hard surface sanitation. In one particular embodiment, the disinfectant composition can be mixed with natural aloe gel to form a disinfectant aloe formulation. Such a formulation would be useful for application to burns, skin infections, and other irritations. The aloe may act as a thickening agent, or may also include another thickening or gelling agent as described above, depending on the desired viscosity of the disinfectant gel.

In another embodiment, a disinfectant composition comprising one or more digestive enzymes can be formulated into a disinfectant foam or foaming composition. The disinfectant foams or foaming compositions include the disinfectant composition and foaming agents. Any foaming agent known in the art can be used depending on the desired application and characteristics of the resulting disinfectant foam. As with the disinfectant composition, the disinfectant foams of the present invention can be used in both human (e.g. hand washing) and industrial applications.

In another embodiment, the disinfectant composition comprising one or more digestive enzymes can be in the form of a disinfectant aerosol or fog. Fogging, also referred to as thermal fogging, is the process by which disinfectants are aerosolized.

The aerosol particles of the disinfectant are suspended within the air for a period of time in order to disinfect both the air itself and surfaces, including inaccessible parts of a structure such as air vents. The aerosolized particles of disinfectant can have a particle size of from about 5 μm to about 200 μm. In another embodiment, the aerosolized particle can have a particle size of from about 20 μm to about 150 μm.

Methods for evaluating the disinfectant ability of a particular composition are known to those having ordinary skill in the art. Typically, the relative effectiveness of a disinfectant can be measured by comparing how well it disinfects as compared to a known disinfectant. Phenol is one known disinfectant standard, and the corresponding rating system is called the "Phenol coefficient". The disinfectant to be tested is compared with phenol on a standard microbe, e.g., *E. coli* or *S. aureus*. Disinfectants that are more effective than phenol have a coefficient > 1. Those that are less effective have a coefficient < 1. To calculate phenol coefficient, the concentration of the test compound at which the compound kills the test organism in 10 minutes, but not in 5 minutes, is divided by the concentration of phenol that kills the organism under the same conditions. The phenol coefficient may be determined in the presence of a standard amount of added organic matter or in the absence of organic matter. One particular phenol coefficient assay uses the Rideal-Walker method. The U.S. Department of Agriculture also has a method that gives a U.S. Department of Agriculture coefficient. Other methods are known to those having ordinary skill in the art.

A disinfectant as described herein can have a phenol coefficient for *S. aureus* that is > 1, e.g., greater than 1.05, 1.1, 1.2, 1.3, 1.4, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, or higher. In some cases, the phenol coefficient is in the range from about 2 to about 20, e.g., about 4 to about 10, about 2 to about 6, about 6 to about 12, or about 10 to about 15.

A disinfectant as described herein can have a phenol coefficient for *E. coli* that is > 1, e.g., greater than 1.05, 1.1, 1.2, 1.3, 1.4, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18. In some cases, the phenol coefficient is in the range from about 2 to about 20, e.g., about 4 to about 10, about 2 to about 6, about 6 to about 12, or about 10 to about 15.

A disinfectant or sanitizer as described herein can be bacteriocidal and/or bacteriostatic to *E. coli*. In some embodiments, a disinfectant or sanitizer as described herein can be bacteriocidal and/or bacteriostatic against STEC, ETEC, or EPEC, or any 2, or all 3.

Detergents

A disinfectant composition comprising one or more digestive enzymes described herein can also be formulated as a detergent. A detergent is a material intended to assist cleaning. A detergent can contain one or more digestive enzymes, as described previously, in a formulation suitable to maintain its disinfectant ability, and can contain optional active or inactive ingredients, e.g., enzyme stabilizers, additional disinfectants, bleaches, soaps, surfactants, colorants and perfumes, abrasives, pH modifiers, acids, alkalis, or caustic compounds, water softeners, oxidants, suspending agents, fabric softeners, foaming agents or anti-foaming agents, viscosity modifiers, corrosion inhibitors, and optical brighteners. A detergent as described herein can be bacteriostatic and/or bacteriocidal to *E. coli*, and in some embodiments can be bacteriostatic and/or bacteriocidal against STEC, ETEC, or EPEC, or any 2, or all 3.

A detergent composition comprising one or more digestive enzymes, especially those made for use with water, can include additional components such as surfactants to 'cut' (dissolve) grease and to wet surfaces, abrasives to scour, substances to modify pH or to affect performance or stability, acids for descaling or caustics to break down organic compounds, water softeners to counteract the effect of "hardness" ions, oxidants (oxidizers) for bleaching, disinfection, and breaking down organic compounds, non-surfactant materials that keep dirt in suspension, enzymes to digest proteins, fats, or carbohydrates in stains or to modify fabric feel, ingredients that modify the foaming properties of the cleaning surfactants, to either stabilize or counteract foam, ingredients to increase or decrease the viscosity of the solution, or to keep other ingredients in solution, in a detergent supplied as a water solution or gel, ingredients that affect aesthetic properties of the item to be cleaned, or of the detergent itself before or during use, such as optical brighteners, fabric softeners, colors, and perfumes, ingredients such as corrosion inhibitors to counteract damage to equipment with which the detergent is used, ingredients to reduce harm or produce

benefits to skin, when the detergent is used by bare hand on inanimate objects or used to clean skin, and preservatives to prevent spoilage of other ingredients.

The detergent composition may be in any convenient dry form, e.g., a bar, a tablet, a powder, a granule or a paste. It may also be a liquid detergent.

The digestive enzyme(s) of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g., a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, and the composition may be formulated as described in e.g. WO 92/19709 and WO 92/19708.

Antiseptics

Various embodiments of compositions comprising on or more digestive enzymes can also be used as antiseptic agents, e.g., to reduce, eradicate, or attenuate *E. coli* on skin or other living tissues. Antiseptics are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. They should generally be distinguished from antibiotics that destroy bacteria within the body, and from disinfectants, which destroy microorganisms found on non-living objects. Some antiseptics are true germicides, capable of destroying microbes (bacteriocidal), while others are bacteriostatic and only prevent or inhibit their growth.

The presently described antiseptics can find particular use in hospital or healthcare settings, e.g., in hand, facial or body-wash formulations; as antiseptics for use prior to and post surgical treatment; and as antiseptics for use in cleansing and treating wounds, such as traumatic or surgical wounds. In the community setting, antiseptics are useful in any setting where community-acquired infections are of concern, e.g., daycare settings, large institutions, schools, etc. Antiseptics can also be useful in the home setting in hand, facial or body-wash formulations, or for treating wounds.

An antiseptic can include one or more digestive enzymes, in various embodiments as described previously, and can optionally include one or more active or inactive ingredients, such as other antiseptic agents known to those having ordinary skill in the art, stabilizers (e.g., enzyme stabilizers), colorants, perfumes, and other

excipients. Examples of antiseptic agents to include with the one or more digestive enzymes include alcohols (e.g., ethanol, 1- and 2-propanol, or mixtures thereof), quaternary ammonium compounds (benzalkonium chloride, cetyl trimethylammonium bromide, cetylpyridinium chloride, and benzethonium chloride), boric acid, chlorhexidine gluconate, peroxides (e.g., hydrogen peroxide, benzoyl peroxide); iodine and iodophor solutions (e.g., povidone-iodine), octenidine dihydrochloride, Phenolic (carbolic acid) and phenolic derivative compounds, sodium chloride, sodium hypochlorite, and calcium hypochlorite.

An antiseptic as described herein can be bacteriocidal and/or bacteriostatic to *E. coli*, and in some embodiments can be bacteriocidal and/or bacteriostatic to STEC, ETEC, or EPEC, or any 2, or all 3.

In one embodiment, an antiseptic composition comprises one or more digestive enzymes and optionally one or more of an anti-inflammatory agent, an analgesic, or an anaesthetic.

An anti-inflammatory agents can include steroidal and non-steroidal anti-inflammatory compounds. In one embodiment, the antiseptic composition includes one or more steroids. In one embodiment, the antiseptic compositions can comprise an anti-inflammatory agent that is a non-steroidal anti-inflammatory drug. Non-limiting examples of suitable non-steroidal anti-inflammatory drugs include aspirin (Anacin, Ascriptin, Bayer, Bufferin, Ecotrin, Excedrin), choline and magnesium salicylates (CMT, Tricosal, Trilisate), choline salicylate (Artliropan), celecoxib (Celebrex), diclofenac potassium (Cataflam), diclofenac sodium (Voltaren, Voltaren XR), diclofenac sodium with misoprostol (Arthrotec), difiunisal (Dolobid), etodolac (Lodine, Lodine XL), fenoprofen calcium (Nalfon), flurbiprofen (Ansaid), ibuprofen (Advil, Motrin, Motrin IB, Nuprin), indomethacin (Indocin, Indocin SR), ketoprofen (Actron, Orudis, Orudis KT, Oruvail), magnesium salicylate (Arthritab, Bayer Select, Doan's Pills, Magan, Mobidin, Mobogesic), meclofenamate sodium (Meclomen), mefenamic acid (Ponstel), meloxicam (Mobic), nabumetone (Relafen), naproxen (Naprosyn, Naprelan), naproxen sodium (Aleve, Anaprox), oxaprozin (Daypro), piroxicam (Feldene), rofecoxib (Vioxx), salsalate (Amigesic, Anaflex 750, Disalcid, Marthritic, Mono-Gesic, Salflex, Salsitab), sodium salicylate, sulindac (Clinoril), tolmetin sodium (Tolectin), valdecoxib (Bextra), or a combination thereof.

Non-limiting examples of analgesics include acetylsalicylic acid, codeine, ibuprofen, acetaminophen, or tea tree oil. Non-limiting examples of anaesthetics include xylocaine, prilocaine or benzocaine.

Exemplary methods of testing candidate antiseptic compositions are provided below. One skilled in the art will understand that other methods of testing the antiseptic compositions are known in the art and are also suitable for testing candidate antiseptic compositions.

In vitro methods of determining the ability of candidate antiseptic compositions to kill or inhibit the growth of microbial cells such as *E. coli* are well-known in the art. In general, these methods involve contacting a culture of the cells of interest with various concentrations of the candidate antiseptic compositions and monitoring the growth of the cell culture relative to an untreated control culture. A second control culture comprising cells contacted with a known anti-microbial agent may also be included in such tests, if desired.

For example, the ability of a candidate antiseptic composition to inhibit the growth of microbial cells can readily be determined by measurement of the minimum inhibitory concentration (MIC) for the antiseptic composition. The MIC is defined as the lowest concentration that inhibits growth of the organism to a pre-determined extent. For example, a MIC_{100} value is defined as the lowest concentration that completely inhibits growth of the organism, whereas a MIC_{90} value is defined as the lowest concentration that inhibits growth by 90% and a MIC_{50} value is defined as the lowest concentration that inhibits growth by 50%. MIC values are sometimes expressed as ranges, for example, the MIC_{100} for an antiseptic composition may be expressed as the concentration at which no growth is observed or as a range between the concentration at which no growth is observed and the concentration of the dilution which immediately follows.

Anti-bacterial MICs for candidate antiseptic compositions can be measured using a broth macro- or microdilution assay (see Amsterdam, D. (1996) "Susceptibility testing of antimicrobials in liquid media," pp.52-111. In Loman, V., ed. Antibiotics in Laboratory Medicine, 4th ed. Williams and Wilkins, Baltimore, MD). A standardised anti-bacterial susceptibility test is provided by the National Committee for Clinical Laboratory Standards (NCCLS) as NCCLS, 2000; document M7-A58.

In the classical broth microdilution method, the candidate antiseptic composition is diluted in culture medium in a sterile, covered 96-well microtiter plate. An overnight culture of a single bacterial colony is diluted in sterile medium such that, after inoculation, each well in the microtiter plate contains an appropriate number of colony forming units (CFU)AnI (typically, approximately 5 x 105 CFU/ml). Culture medium only (containing no bacteria) is also included as a negative control for each plate and known antibiotics are often included as positive controls. The inoculated microtiter plate is subsequently incubated at an appropriate temperature (for example, 35°C - 37°C for 16-48 hours). The turbidity of each well is then determined by visual inspection and/or by measuring the absorbance, or optical density (OD), at 595nm or 600nm using a microplate reader and is used as an indication of the extent of bacterial growth..

Anti-microbial effects may also be expressed as the percentage (%) inhibition of growth of a given micro-organism over a pre-determined period of time by treatment with a single concentration of a candidate antiseptic composition. This method provides a rapid method of assessing the ability of an antiseptic composition to inhibit microbial growth, for example, prior to conducting more in-depth tests, such as MlC determinations or *in vivo* testing.

The ability of any of the present disinfectant, detergent, santitizer, and antiseptic compositions to kill or inhibit the growth of *E. coli* bacteria can be tested using methods well-known in the art, including the various methods described above. Methods and protocols for testing compositions against specific bacteria can be found, for example, in Official Methods of Analysis of the AOAC, 15th Ed., Arlington Virginia 22201, USA (Association of Official Analytical Chemists (AOAC), Inc. 1990), Designation: E 1054-91 "Practices for Evaluation Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products" (American Society for Testing and Materials (ASTM), 1991). As is also known in the art, *in vitro* Time-Kill evaluations can be performed using a modification of the methods described in the Draft European Standard, prEN 12054, "Chemical Disinfectants and Antiseptics - Products for Hygienic and Surgical Handrub and Handwash - Bactericidal Activity - Test Method and Requirements (1995)." Additional methods that may be used include the log reduction test, proliferation testing, the AOAC use

dilution test, or the zone of inhibition test. Other methods are described in the Examples below.

Kits

Also provided herein are kits. Typically, a kit includes one or more compositions as described herein. In certain embodiments, a kit can include one or more delivery or administration systems, e.g., for delivering or administering a composition as provided above, and/or directions for use of the kit (e.g., instructions for treating a patient; instructions for disinfecting a surface). In another embodiment, the kit can include a composition as described herein and a label, e.g., a label that indicates that the contents are to be administered to a patient with a *E. coli* infection, or a label as to how to use the composition as a disinfectant, sanitizer, detergent, or antiseptic.

Methods of Use

Pharmaceutical compositions (e.g., antibiotic compositions) described previously can be used to treat or prevent *E. coli* infections in animals, e.g., mammals and birds. In particular, the pharmaceutical compositions can be used to ameliorate one or more symptoms and side effects of such infections and/or to reduce or eradicate the *E. coli* bacterium causing the infection. The pharmaceutical compositions can be in any appropriate dosage form, as described previously. In certain embodiments, the antibiotic compositions described herein are used to treat wounds or lesions that have become infected, e.g., wounds resulting from trauma or surgery. Such use can reduce scarring and promote wound healing in patients having infected wounds. Compositions formulated for pharmaceutical use can also be employed prophylactically, e.g., as antiseptics. Such compositions find particular use in the prophylactic treatment of surgical incisions and other wounds, to prevent *E. coli* infection.

The digestive enzymes provided herein can be used to treat a variety of diseases and disorders associated with infection by *E. coli* bacteria or in which *E. coli* bacteria are implicated. Certain embodiments include infections associated with medical devices or prostheses, e.g. catheter, grafts, prosthetic heart valves, artificial

joints, etc. In some embodiments, a composition comprising one or more digestive enzymes can be coated onto the medical device either at manufacture of the device or after manufacture but prior to insertion of the device.

Surgical wounds, especially those associated with foreign material, e.g. sutures may also be treated with the compositions provided herein. As many as 71% of all nosocomial infections occur in surgical patients, 40% of which are infections at the operative site. Despite efforts to prevent infection, it is estimated that between 500,000 and 920,000 surgical wound infections complicate the approximately 23 million surgical procedures performed annually in the United States.

The digestive enzymes alone or with an antibiotic, anesthetic, or anti-inflammatory may be applied as an ointment, cream or liquid to the wound site or as a liquid in the wound prior to and during closure of the wound. Following closure, a composition comprising one or more digestive enzymes could be applied at dressing changes. For wounds that are infected, the composition could be applied topically and/or systemically.

In early acute onset of osteomyelitis the vascular supply to the bone is compromised by infection extending into surrounding tissue. Within this necrotic and ischemic tissue, the bacteria may be difficult to eradicate even after an intense host response, surgery, and/or antibiotic therapy. The main organisms responsible are SA and *E. coli*.

The digestive enzymes could be administered systemically alone or in combination with other antibiotics. Treatment could be 2-6 weeks in duration. The antibiotic could be given as a continuous infusion or multiple administration during the day. A composition comprising one or more digestive enzymes could be used as an antibiotic-impregnated cement or as antibiotic coated beads for joint replacement procedures.

Treatment or prevention of sepsis in immunocompromised host is also provided. Treatment of infections in patients who are immunocompromised by virtue of chemotherapy-induced granulocytopenia and immunosuppression related to organ or bone marrow transplantation represents a significant challenge. The neutropenic patient is especially susceptible to bacterial infection, so antibiotic therapy should be initiated promptly to cover likely pathogens, if infection is suspected. Organisms likely to cause infections in granulocytopenic patients are: SA and *E. coli*.

The digestive enzyme composition alone or with an antibiotic is preferably administered orally or systemically for 2-6 weeks in duration. The digestive enzymes could be given as a continuous infusion or via multiple administrations during the day.

Disinfectant, sanitizing, and detergent compositions as described herein can be applied to non-living surfaces in the appropriate amounts and manner to reduce or eradicate *E. coli* on such surfaces, and thus can reduce or prevent *E. coli* transmission and/or infectivity. Concentrations, timing, and frequency of treatment are parameters that can be determined by one having ordinary skill in the art.

Any surface can be disinfected with the described compositions, including a variety of medical devices used in the hospital or health-care setting. As used herein, "medical device" refers to any device for use in or on a patient, such as an implant or prosthesis. Such devices include, without limitation, synthetic vascular grafts, blood monitoring devices, artificial heart valves, scalpel, knife, scissors, spatula, expander, clip, tweezer, speculum, retractor, suture, surgical mesh, chisel, drill, level, rasp, saw, splint, caliper, clamp, forceps, hook, lancet, needle, cannula, curette, depressor, dilator, elevator, articulator, extractor, probe, staple, valve, catheter, stent, tubing, bowl, tray, sponge, snare, spoon, syringe, pacemaker, screw, plate, or pin.

Other community and hospital or health-care surfaces suspected of harboring *E. coli* can be disinfected, including large or small surfaces (floors, tables, changing tables, beds, ventilation systems, tubs, door handles, counters, food service surfaces, etc.). The compositions can also find use in hand or body washes, e.g., at points of entry to community settings, hospital rooms, or bathrooms.

The compositions provided herein may be used in the manner of common disinfectants or in any situation in which microorganisms are undesirable. For example, they may be used as surface disinfectants, in coatings for medical devices, in coatings for clothing, such as to inhibit growth of bacteria or repel mosquitoes, in filters for air purification, such as on an airplane or in community or hospital settings, in water purification system, as constituents of shampoos and soaps, as food preservatives, cosmetic preservatives, media preservatives, in herbicides or insecticides, as constituents of building materials, such as in silicone sealant, and in animal product processing, such as the curing of animal hides or in slaughterhouses.

For these purposes, typically the digestive enzymes alone or in conjunction with other disinfectants or detergents are included in the compositions and applied with an appropriate applicator. They also may be incorporated or impregnated into the material during manufacture, such as for an air filter, or otherwise applied to the material or the object.

For example, in some embodiments, a composition described herein can be mixed in with the material, for example during manufacturing of the material or at a subsequent time. In addition, a composition can be applied to the surface of a material, either during manufacturing or at a subsequent time. As used herein, the term "suitable material" means any material on, to, or in which the digestive enzymes can be applied or incorporated, thereby incorporating an antimicrobial activity in/on the material. For example, a gauze pad on a bandage can be manufactured with a composition comprising one or more digestive enzymes in or on the gauze, and/or an ointment comprising one or more digestive enzymes can be applied to the gauze thereby incorporating antimicrobial activity to the gauze. Examples of suitable materials in which digestive enzymes may be used, include, but are not limited to: foods, liquids, a medical deivce (e.g. surgical instruments), a bead, a film, a monofilament, an unwoven fabric, sponge, cloth, a knitted fabric, a short fiber, a tube, a hollow fiber, an artificial organ, a catheter, a suture, a membrane, a bandage, and gauze. The digestive enzymes may be applied or mixed into numerous other types of materials that are suitable for use in medical, health, food safety, or environmental cleaning activities.

Veterinary Applications

The compositions described herein, in pharmaceutical or disinfectant/sanitizer, detergent or antiseptic formats, can also be used in a variety of veterinary applications. For example, many mammals, including dogs, cats, and cows, can be infected with *E. coli* or act as carriers of the bacteria. Accordingly, the present compositions can be used to treat animals infected with or suspected to carry *E. coli* in order to treat the infection or to prevent transmission to other animals, including humans. Disinfectant and detergent compositions can be used to treat animal living quarters and equipment that comes into contact with the animals, while antiseptics and antibiotic formulations can be used to treat animals to prevent or treat infection.

For example, the digestive enzymes provided herein can be used for the prevention and treatment of mastitis, particularly mastitis in dairy cattle, though any mastitis can be treated using the digestive enzymes provided herein. Mastitis in dairy cattle is an inflammation of the mammary gland in response to intramammary bacterial infection, mechanical trauma, or chemical trauma. It is thought that contagious mastitis is primarily caused by *S. aureus* and *Streptococcal agalactiae*. Environmental mastitis can be caused by a variety of different bacteria, including, but not limited to, *K. pneumoniae, E. coli, Klebsiella oxytoca, Enterobacter aerogenes, Streptococcal uberis, Streptococcal bovis*, and *Streptococcal dysgalactia*.

In some embodiments, prevention of bovine mastitis can include daily teat-dipping with a solution comprising one or more digestive enzymes. In some embodiments, the solution comprising one or more digestive enzymes may further include one or more additional antibiotics. When infection does occur, intramammary infusion of one or more digestive enzymes may be implemented. As above, additional antibiotics may also be administered in conjunction with the digestive enzymes. Typically, the digestive enzymes are administered by intramammary injection; however, effective dosages may be administered parenterally, percutaneously, by implant and also by dipping. In some embodiments, bovine mastitis can be treated by administering an effective amount of one or more digestive enzymes to a cow. The administration may be a prophylactic administration, in that all cattle in the herd are treated with a digestive enzyme composition, or the administration may occur when infection occurs in individual cows.

Introduction of *S. aureus* (also referred to as SA herein) bacteria and *E. coli* bacteria can occur during the preparation of beef, poultry, fish and pork products. Accordingly, in some embodiments, reduction of infection can be provided through administration of one or more digestive enzymes to an animal (e.g., cow, chicken, turkey, fish, or pig) to reduce the presence of *E. coli* or *SA* bacteria in the intestines of the animal. Administration may occur through any available method including injection and through the introduction of one or more digestive enzymes in feed.

In some embodiments, administration of one or more digestive enzymes to an animal may be used to prevent or reduce transmission of SA bacteria or *E. coli* from

the animal to other animals or humans. Administration of the digestive enzymes may be accomplished through any available method known in the art.

Food Applications

Also provided herein is a method of preventing SA or *E. coli* infection of beef, poultry, fish, and pork. Beef processing is a common point of contamination: during the slaughtering process, the contents of intestines or fecal material on the hide could mix with the meat, thus allowing bacteria to flourish in the warm, damp conditions. If the infected parts are then ground, the bacteria go from the surface of the cut to the interior of the ground mass. Additionally, in the production of ground beef, meat from multiple cattle is often ground together, enabling contamination from a single animal to infect an entire lot of ground beef. Accordingly, in some embodiments, reduction of infection can be provided through administration of one or more digestive enzymes to a cow to reduce the presence of bacteria in the intestines of the cow. Administration may occur through any available method including injection and through the introduction of one or more digestive enzymes in feed. In another embodiment, the reduction of contamination of meat during slaughter and grinding can be provided through the use of sprays containing one or more digestive enzymes as provided herein. Such sprays may be used, for example, in the disinfection of slaughter and grinding instruments or in the disinfection of the ground meat itself. The methods described above can further be used during the slaughter and preparation of poultry, fish and pork products (e.g., through administration or one or more digestive enzymes to poultry, fish and/or pigs prior to slaughter).

E. coli and SA bacteria can also be spread through unwashed fruits and vegetables. Accordingly, also provided herein is a method of washing fresh fruits and vegetables using a solution, wash, aerosol, fog, gel, or powder comprising one or more digestive enzymes as provided herein. A produce wash is a solution used to bathe the surface of produce, and typically is in contact with the produce from about 30 sec to about 5 min. A produce soak is a solution in which produce items are immersed for a time from about 30 sec to about 30 min. However, the terms and solutions can be used interchangeably unless otherwise distinguished. It is understood that the temperature at which produce is washed or soaked will influence the length of time necessary to reduce or inactivate bacteria thereon, with warmer temperatures leading to a shorter time necessary for treatment.

The wash or soak solutions described herein can be used to reduce bacterial number, especially bacterial pathogen number, on the surfaces of fruits, vegetables, raw cut meat products, fish, shellfish, at the consumer level (in the household), in commercial food preparation environments, on fruits and/or vegetables prior to juicing, by wholesale or retail operators, and/or at the level of the harvest, meat packing plant or slaughterhouse, fishing boat, and so on, without limitation. The present methods are particularly useful for inactivating *E. coli* on the surfaces of fresh fruits and vegetables.

Methods for Evaluating Activity

Compositions described herein can be evaluated for a variety of activities by methods known to those having ordinary skill in the art. For example, enzymatic activities can be evaluated using standard enzyme assays. Minimum Inhibitory Concentrations (MIC) of the compositions can also be evaluated by methods known to those having ordinary skill in the art, as described above. Other assays, including the Phenol coefficient, are also well known to the skilled artisan. *See also* the Examples below.

EXAMPLES

Exemplary Liquid Compositions

A dry pancreatic enzyme composition containing about 200 USP units/mg of protease, about 40 USP units/mg of lipase, and about 250 USP units/mg of amylase can be diluted with various diluents (water, saline, phosphate buffered solutions, pH stabilized solutions) and optionally with other active or inactive additives (enzyme stabilization systems, buffers, colorants, sanitizers, detergents, disinfectants, antiseptics) to form exemplary liquid compositions for the uses described herein. In some embodiments, the dry enzyme composition can be diluted in a ratio of mgs of the dry enzyme composition to mls of the total diluent in the range from 1 mg enzyme composition:1 ml total diluent to 1 mg enzyme composition:10,000 mls total diluent, or any value in-between, e.g., 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:50, 1:100, 1:200, 1:500, 1:1000, 1:5000, or 1:10,000.

Exemplary Solid Compositions

A dry pancreatic enzyme composition containing about 200 USP units/mg of protease, about 40 USP units/mg of lipase, and about 250 USP units/mg of amylase can be mixed with various active or inactive dry ingredients and additives (e.g., dry detergents, disinfectants, antiseptics, and sanitizers, such as alkyl ethoxylate sulface, SDS, sodium laureth sulfate, dodecylbenzene, sodium 4-dodecylbenzenesulfonate, enzyme stabilization systems, excipients, colorants) to form exemplary solid compositions. In some embodiments, the dry enzyme composition can be mixed with total mgs of the additives in the range from 1 mg enzyme composition:1 mg total additives to 1 mg enzyme composition:10,000 mgs total additives, or any value in-between, e.g., 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:50, 1:100, 1:200, 1:500, 1:1000, 1:5000, or 1:10,000.

Exemplary Topical Formulations

A dry pancreatic enzyme composition containing about 200 USP units/mg of protease, about 40 USP units/mg of lipase, and about 250 USP units/mg of amylase can be mixed with various carriers appropriate for topical pharmaceutical formulations in ratios ranging from about 1 mg of the enzyme composition to 1 mg of the carrier to about 1 mg of the enzyme composition to about 200 mg of the carrier, or any ratio therebetween (e.g., 1:2, 1:4, 1:5, 1:10, 1:20, 1:30, 1:40, 1:50, 1:75, 1:100, 1:150). For example, in one embodiment a 1:25 ratio is employed, and the carrier is petrolatum.

Bacterial Limit Testing- Evaluation of Bacteriocidal and Bacteriostatic Properties

The present inventors have surprisingly found in the course of doing bacterial limit testing on a dry pig pancreatic enzyme composition comprising about 200 USP units/mg protease activity, about 40 USP units/mg of lipase activity, and 250 USP units/mg of amylase activity that *E. coli* bactera do not grow in the presence of various dilutions of this material. Recovery of *E. coli* CFUs from positive controls "spiked" with *E. coli* was consistently low to absent. Dilution bacterial limit testing of the dry composition in both unencapsulated and lipid-encapsulated (20% soy oil by weight of the composition) form demonstrate minimal to no recovery of the bacterium from positive controls to which a known number of CFUs of *E. coli* had been added.

A lack of recovery during such recovery methods suggests a bacteriostatic and/or bacteriocidal nature of the composition.

Methods and Materials

Sample materials - Unencapsulated Porcine Pancreatic Enzyme Concentrate (uPEC) isolated from the pig (Sus scrofa) was manufactured by a commercial supplier (Scientific Protein Labs) to contain approximately 200 U/mg protease activity, 40 U lipase activity/mg, and 250 U of amylase activity/mg. A lipid encapsulated version of this material (ePEC) was obtained by using a modified fluidized bed process and a fully hydrogenated highly purified organic oil (fully hydrogenated soy oil) which was then used to coat the enzyme particles at a percentage by weight of about 20% of the final particles.

Methods: Both the ePEC and uPEC underwent standard microbial analysis for the detection of microbes. Both compositions failed to show any significant contamination using Microbial Limits Testing per USP methods. Additionally, both samples were negative/10g for *Salmonella* species and *E. coli*. Briefly, the procedure for the examination of test compositions for microbiological suitability (i.e., estimation of the total number of viable microorganisms and freedom from specific organism) is outlined in the USP, chapter 61, "Microbial Limit Tests." USP 61 outlines preparatory testing, during which is determined the test parameters wherein the test composition itself no longer inhibits the multiplication of viable organisms. USP 1227 ("Validation") also provides guidance to validate recovery methods. Methods for evaluating Total Aerobic Microbial Count, *E. Coli*, and *Salmonella* species were performed.

Total Aerobic Microbial Count: *Preparation of test dilutions* - Bulk dilutions of ePEC and uPEC were prepared at 1:10, 1:50, 1;100 and 1:200 in Tryptic Soy broth containing 4% Polysorbate 20 and 0.5% lecithin. The bulk test sample dilution was then split into separate 10 ml aliquots, where were then inoculated with a low number of colony forming units (CFUs <100/mL) of the appropriate microorganisms (*S. aureus, P. aeruginosa, E. coli, S. enterica*). One millimeter of the inoculated aliquots was plated in duplicate using the appropriate solid medium (agar). Positive controls

were prepared, inoculated, and plated in a manner similar to the test samples. Negative controls were prepared, inoculated with sterile reagent, and plated in a manner similar to the test samples. The plates were incubated at 30°C to 35°C for two days. At the end of the period the recovery was calculated. Recovery of inoculated organisms must be at least 70% of the positive control in order to show no inhibition of growth by the test composition. Triphenyltetrazolium chloride was used to count the plates.

Test for Salmonella species - Dilutions prepared with Lactose broth containing 4% polysorbate 20 and 0.5% lecithin were inoculated with <= 100 CFU of Salmonella enterica. The inoculated dilutions were incubated at 30°C to 35°C for 24 hours prior to 1 ml being transferred to both Selenite Cystine and Tetrahionate broths. The selective broths were incubated for 18 hours at 30°C to 35°C prior to being streaked to brilliant green, bismuth sulfite an xylose lysine deoxycholate agars. The selective agar plates were incubated at 30°C to 35°C for 24 hours. The plates were observed for colonies characteristic of Salmonella species. Where observed, a representative colony was confirmed to be *Salmonella* species using an API 20e biochemical identification test.

Test for E. coli - Dilutions prepared with Lactose broth containing 4% polysorbate 20 and 0.5% lecithin were inoculated with <= 100 CFU of *E. coli*. The inoculated dilutions were incubated at 30°C to 35°C for 24 hours prior to being streaked to MacKonkey agar. The plates were observed for colonies characteristic of E. coli. Where observed, a representative colony was confirmed to be *E. coli* using an API 20e biochemical identification test.

Results

Total Aerobic Count: Test Results using the uncoated PEC (uPEC) are shown in **Table 1**, below. As shown, for dilutions of 1:50, 1:100, and 1:200 the percent recovery of *S. aureus* was 0%, demonstrating the bacteriocidal and/or bacteriostatic action of uPEC of *S. aureus*.

Table 1

Recovery of Microorganisms following Incubation with uPEC					
Organism	Negative Control	Positive Control	Dilution	Test Average	% Recovery
S. Aureus	0 CFU	57 CFU	1:50	0 CFU	0%
			1:100	0 CFU	0%
			1:200	0 CFU	0%
E. coli	0 CFU	63 CFU	1:50	0 CFU	0%
			1:100	1 CFU	2%
			1:200	1 CFU	2%
S. enterica	0 CFU	66 CFU	1:50	48 CFU	73%
			1:100	62 CFU	94%
			1:200	56 CFU	85%

The uPEC percent recovery for *E. coli* is also given in **Table 1**. For a dilution of 1:50 the recovery was 0%, for a dilution of 1:100 the recovery was 2%, and for a dilution of 1:200 the recovery was 2%.

The uPEC percent recovery for *S. enterica* is also given in **Table 1**. For a dilution of 1:50 the recovery was 73%, for a dilution of 1:100 the recovery was 94%, and for a dilution of 1:200 the recovery was 85%.

Test results using the lipid encapsulate PEC (ePEC) are given in **Table 2**, below. As shown, for dilutions of 1:50 the recovery of *S. aureus* was 29%, for a dilution of 1:100 the recovery was 0%, and for a 1:200 the percent recovery of *S. aureus* was also 0%.

Table 2

Recovery of Microorganisms following Incubation with ePEC					
Organism	Negative Control	Positive Control	Dilution	Test Average	% Recovery
S. aureus	0 CFU	34 CFU	1:50	10 CFU	29%
			1:100	0 CFU	0%
			1:200	0 CFU	0%
E. coli	0 CFU	38 CFU	1:50	12 CFU	32%
			1:100	9 CFU	17%
			1:200	28 CFU	78%
S. enterica	0 CFU	61 CFU	1:50	53 CFU	87%
			1:100	N/P	N/P
			1:200	N/P	N/P

The ePEC percent recovery for *E. Coli* are also in **Table 2**. For a dilution of 1:50 the recovery was 32%, for a dilution of 1:100 the recovery was 17%, and for a dilution of 1:200 the recovery was 78%.

The ePEC percent recovery for *S. enterica* are also given in **Table 2**. For a dilution of 1:50 the recovery was 87%, for a dilution of 1:100 and 1:200 the tests were not performed.

Positive controls as reported in **Tables 1** and **2** clearly demonstrate that the growth media for all microbiological cultures were functioning effectively. In **Table 1**, it can be seen that the uPEC composition was highly effective on both *S. aureus* and *E. coli*. This is substantiated by the fact that recovery of positively spiked controls failed to meet the USP criterion of positive recovery (at least 70% of spiked sample CFUs recovered).

It is also important to note that the bacteriostatic/bactericidal activity showed species specificity. Samples of *S. enterica* showed excellent recovery using both coated and

uncoated PEC. Because bacteria share common cell wall and membrane structures such as lipids and peptidoglycans, it is possible that these results point to the sensitivity of a more species specific cellular component such as a protein. If lipase or amylase action alone were sufficient to induce bacterial death or suppression of growth, then it would be unlikely to see the recovery of *S. enterica* at such robust levels. It is possible that species specific protein(s) in *S. aureus* and *E. coli* share similar peptide sequences and the appropriate local tertiary structure to allow for enzymatic attack by one or several of the proteases present in PEC, leading to a subsequent destruction of the bacterium. These results do not however, rule out a multi-staged event where action by lipases and amylases against the bacterial cell wall and membrane first expose extracellular or transmembrane proteins which have the appropriate primary and tertiary structure to lend themselves to enzymatic degradation by one or many of the PEC proteases.

These results demonstrate a clear bacteriostatic effect on *S. aureus* and *E. coli* for both lipid coated and uncoated PEC. Because this experiment relies upon an end point read out of bacterial colony growth while the subject bacteria remain in the presence of PEC, it is not possible on the basis of these results to rule out the possibility of a bacteriostatic effect only. Thus, failure to recover a sufficient number of viable colony forming units could be the result of the continued suppressive presence of the PEC material, as opposed to the induction of bacterial death. Consequently, additional experiemental testing was carried out in order to more conclusively evaluate the bactericidal capabilities of PEC.

Materials and Methods: The uncoated PEC formulation described above previously was used to evaluate bacteriocidal activity of the formulations.

Preparation of test dilutions - Bulk dilutions of uPEC were prepared at 1:100 and 1:200 in Tryptic Soy broth containing 4% Polysorbate 20 and 0.5% lecithin. The bulk dilution was then split into separate 10 ml aliquots, where were then inoculated with a low number of colony forming units (CFUs <100) of the appropriate microorganisms. One millimeter of the inoculated aliquots was plated in duplicate using the appropriate solid medium (agar). Positive controls were prepared, inoculated, and plated in a manner similar to the test samples. Negative controls were

prepared, inoculated with sterile reagent, and plated in a manner similar to the test samples. The plates were incubated at 30°C to 35°C for two days. Following this initial incubation, cultured material was then collected and washed in Phosphate Buffered Saline (PBS) and filtered in order to remove PEC. The material was resuspended in diluted in Tryptic Soy broth as per above and re-plated onto fresh solid (agar) media and incubated for an additional 2 days. At the end of this period, colonies were enumerated and the percentage recovery calculated. At the end of the period the recovery was calculated. Recovery of inoculated organisms must be at least 70% in order to show no inhibition of growth. Triphenyltetrazolium chloride was used to count the plates.

Tests for E. coli - Dilutions prepared with Lactose broth containing 4% polysorbate 20 and 0.5% lecithin were inoculated with <= 100 CFU of *E. coli*. The inoculated dilutions were incubated at 30°C to 35°C for 24 hours prior to being streaked to MacKonkey agar. The plates were observed for colonies characteristic of *E. coli*. Where observed, a representative colony was confirmed to be *E. coli* using an API 20e biochemical identification test.

The tests were also repeated for both *S. Aureus* and *E. Coli* at Dilutions of 1:20, 1:40, and 1:80.

Test Results

Test Results using the uncoated uPEC are given in **Tables 3** and **4**, below, demonstrating recovery of bacteria following replating of bacteria alone, post-incubation with uPEC and washing. As shown in **Table 3**, for dilutions of 1:100, and 1:200, the percent recovery of *S. aureus* was 13% and 48%, respectively, clearly demonstrating the bacteriocidal action of uPEC on *S. aureus*. As shown in **Table 4**, the percent recovery of *S. Aureus* at a dilution of 1:20 is 2%, for a dilution of 1:40 it is 4%, and for a dilution of 1:80 it is 23%.

Table 3

Bactericidal Action Post Wash / Recovery of Microorganisms					
Organism	Negative Control	Positive Control	Dilution	Test Average	% Recovery
S. Aureus	0 CFU	48 CFU	1:100	6 CFU	13%
			1:200	23 CFU	48%
E. coli	0 CFU	46 CFU	1:100	1 CFU	2%
			1:200	13 CFU	28%

Table 4

Bactericidal Action Post Wash / Recovery of Microorganisms					
Organism	Negative Control	Positive Control	Dilution	Test Average	% Recovery
S. Aureus	0 CFU	47 CFU	1:20	1 CFU	2%
			1:40	2 CFU	4%
			1:80	11 CFU	23%
E. coli	0 CFU	43 CFU	1:20	0 CFU	0%
			1:40	0 CFU	0%
			1:80	5 CFU	12%

The recovery for *E. coli* is also given in **Tables 3** and **4**. As shown in **Table 3**, for a dilution of 1:100 the recovery was 2%, and for a dilution of 1:200 the recovery was 28%. As shown in **Table 4**, the percent recovery of *E. Coli* at dilution of 1:20 is 0%, for a dilution of 1:40 it is 0%, and for a dilution of 1:80 it is 12%

Positive controls demonstrate that the growth media for all microbiological cultures were functioning effectively. This argues for a true bactericidal effect when the selected organisms are exposed to PEC. If the action of the PEC were merely bacteriostatic, removing the PEC from co-culture with the test bacteria would result in

a release from any suppressive action by the PEC with a recovery of active growth. However, removal of PEC does not result in any such recovery, even though cultures were left to grow over a full 24 hr period. This strongly supports the notion that the reason the selected organisms fail to show recovery of CFU's upon exposure to PEC is because of a bactericidal action of the PEC. This action could include mechanisms such as physical and irreversible damage to cell surface lipids, membrane proteins or bacterial capsule by enzymatic degradation, leading to extracellular and intercellular ionic electrolyte imbalances, changes in acidity, and damage to genetic material.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for the treatment or prevention of *E. coli* infection in a bird or a mammal, comprising administering to the bird or mammal a therapeutically effective amount of a pharmaceutical composition comprising one or more digestive enzymes.

2. The method of claim 1 wherein the one or more digestive enzymes comprise one or more enzymes selected from the group consisting of proteases, amylases, celluloses, sucrases, maltases, papain, and lipases.

3. The method of claim 1 wherein the one or more digestive enzymes comprise one or more pancreatic enzymes.

4. The method of claim 1 wherein the one or more of the digestive enzymes comprise pig enzymes.

5. The method of claim 2 wherein the proteases comprise chymotrypsin and trypsin.

6. The method of claim 1 wherein the one or more digestive enzymes are, independently, derived from an animal source, a microbial source, a plant source, a fungal source, or are synthetically prepared.

7. The method of claim 1 wherein the mammal is a pig, horse, cow, dog, cat, monkey, rat, mouse, sheep, goat or human, or the bird is a chicken, duck, turkey, or goose

8. The method of claim 6 where the animal source is a pig pancreas.

9. The method of claim 1 wherein the pharmaceutical composition comprises at least one amylase, a mixture of proteases comprising chymotrypsin and trypsin, and at least one lipase.

10. The method of claim 1 wherein the pharmaceutical composition comprises at least one protease and at least one lipase, and wherein the ratio of total proteases to total lipases (in USP units) ranges from about 1:1 to about 20:1.

11. The method of claim 11 wherein the ratio of proteases to lipases ranges from about 4:1 to about 10:1.

12. The method of claim 1 wherein the pharmaceutical composition is a dosage formulation selected from the group consisting of: pills, tablets, capsules, caplets, sprinkles, creams, lotions, aerosols, emulsions, powders, liquids, gels, and a combination of any thereof.

13. The method of claim 1, wherein the pharmaceutical composition is formulated for oral administration.

14. The method of claim 1, wherein the pharmaceutical composition is formulated for topical administration.

15. The method of claim 1, wherein the pharmaceutical composition is effective against STEC.

16. The method of claim 1, wherein the pharmaceutical composition is effective against ETEC or EPEC.

17. A method of treating a mammal or bird exhibiting one or more symptoms of an *E. coli* infection comprising administering to the mammal or bird a therapeutically effective amount of a composition comprising one or more digestive enzymes.

18. The method of claim 1 or 17, further comprising administering a beta-lactam antibiotic to the mammal or bird.

19. A method for treating diarrhea in an individual, comprising administering a pharmaceutical composition comprising one or more digestive enzymes to the individual.

20. The method of claim 19, wherein the individual is diagnosed with an *E. coli* infection

21. The method of claim 20, wherein the *E. coli* is a STEC.

22. A method for sanitizing or disinfecting a surface to reduce the amount of *E. coli* thereon or to eradicate the *E. coli* thereon, comprising applying to the surface a composition comprising one or more digestive enzymes.

23. The method of claim 22, wherein the surface is a nonliving or inanimate surface.

24. The method of claim 23, wherein the surface is on a medical device.

25. The method of claim 23, wherein the surface is a food.

26. A method for reducing the amount of *E. coli* present on a skin region, tissue, or wound of a mammal or bird comprising applying to the skin region, tissue, or wound a composition comprising one or more digestive enzymes.

27. A disinfectant comprising one or more digestive enzymes, wherein the disinfectant has a phenol coefficient of > 1 to about 20 for *S. aureus* or *E. coli*.

28. An antibiotic comprising one or more digestive enzymes, wherein the antibiotic is bacteriocidal and/or bacteriostatic for *E. coli*.

29. A detergent comprising one or more digestive enzymes, wherein the detergent is bacteriocidal and/or bacteriostatic for *E. coli*.

30. An antiseptic comprising one or more digestive enzymes, wherein the antiseptic is bacteriocidal and/or bacteriostatic for *E. coli*.

31. A disinfectant comprising one or more digestive enzymes, wherein the disinfectant is bacteriocidal and/or bacteriostatic for *E. coli*.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/20259

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 17/00 (2010.01)
USPC - 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C07K 17/00 (2010.01)
USPC - 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/400

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST (PGPB,USPT,USOC,EPAB,JPAB), Google Scholar: protease, $protease, amylase, papain, lipase, $amylase, $lipase, trypsin, $trypsin, digestive enzyme, composition, infection, pharmaceutical, drug, treat, treatment, treating, treated, therap$, administ$, diarrhea, antiseptic, detergent, antimicrobial

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 2006/0105379 A1 (WU et al.) 18 May 2006 (18.05.2006) para [0025]; [0038]; [0149]-[0150]; [0489]; [0723]; [0725]; [1104]	17-21, 26, 28 ---- 1-16
X ---- Y	US 2008/0317731 A1 (GRAMATIKOVA et al.) 25 December 2008 (25.12.2008) para [0590]; [0593]-[0594]; [0598]; [0600]	22-23, 25, 29-31 ---- 24
Y	US 2006/0259995 A1 (CAYOUETTE et al.) 16 November 2006 (16.11.2006) para [0001]; [0011]; [0034]; [0049]-[0050]; [0105]; [0121]; [0132]; [0325]; [0474]-[0476]; [0498]; [0513]-[0515]; [0517]; abstract	1-16, 24, 27
Y	US 2004/0076590 A1 (WILKINS J.) 22 April 2004 (22.04.2004) abstract; para [0015]-[0016]; Tables 1-2	27
Y	US 6,280,726 B1 (WEINRAUCH et al.) 28 August 2001 (28.08.2001) col 2, ln 5-11; col 10, ln 35 -45	8
Y	US 2005/0187130 A1 (BOOKER et al.) 25 August 2005 (25.08.2005) para [0037]	10-11

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 February 2010 (05.02.2010)	05 MAR 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)